How does dual-mycorrhizal association affect the ecological success of kanuka (*Kunzea ericoides*) across the South Island of New Zealand?

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Abstract

In this thesis I investigated the mutualism between *Kunzea ericoides* (kanuka) and two groups of soil fungi, ectomycorrhizae (ECM) and arbuscular mycorrhizae (AMF). Mycorrhizal mutualisms, which are considered globally ubiquitous, are poorly understood over changing abiotic gradients. A field survey of *K. ericoides* assessed how the relationship with the soil fungi varieties altered over a hydrologic gradient. Arbuscular mycorrhizal colonisation was significantly improved by increasing rainfall levels and amount of surrounding kanuka, and negatively affected by increasing altitude. Ectomycorrhizal colonisation was not significantly affected by any measured variables and remained relatively constant across all circumstances, suggesting that it is the preferred fungal mutualist for this tree species.

A glasshouse experiment was done to measure the effect of ECM inoculation on the growth and water usage of *K. ericoides* over varying moisture availability. The seedlings were planted in soil inoculated with both ECM and AMF (experimental) or AMF only (control) and then grown under varying levels of water stress. The experiment was replicated with two soil types, with soil from beneath adult manuka (*Leptospermum scoparium*) and soil from beneath adult kanuka (*K. ericoides*). ECM colonisation significantly increased as soil moisture decreased for both soil types. ECM inoculation also increased the root:shoot ratio, and drastically decreased water usage under drought conditions. There were some soil effects as the seedlings grown in manuka-soil achieving greater biomass than seedlings grown in kanuka-soil. This is possibly due to presence of pathogens or some type of legacy competition which the seedlings would experience growing near conspecifics.

Overall, *K. ericoides* formed a dominant mutualism with ectomycorrhizae. These two both thrive in dry environmental conditions and have a suite of complementing abilities which possibly allow *K. ericoides* to expand it range into these dry habitat types. The increased benefit of these mutualisms at the hydrologic range limit of the species supports the importance of biotic interaction mediating environmental stress. Understanding the effects and response of mycorrhizal mutualisms are especially significant considering current climate change issues in New Zealand and worldwide.

Glossary

Abiotic Factor: the environmental influence exerted by non-living things (e.g. light, rain, etc.)

arbuscule - a highly branched fungal structure occurring within the cortical cells of roots colonized by arbuscular mycorrhizal fungi

Biotic Factor: the environmental influence exerted naturally by living organisms

Ectomycorrhiza (ECM) - a plant-fungus type where the fungal hyphae extend into the plant root and occupy the area between the cortical cells, forming a Hartig net (Sylvia et al. 2005)

Endo- or Arbuscular Mycorrhiza (AMF) - a plant-fungus type in which the fungal hyphae extend into the plant root and occupy the area between and within (intracellularly) the cortical cells (Sylvia et al. 2005). Arbuscule formation is the defining internal structure for arbuscular mycorrhiza.

Facilitation Model of Succession: ecological succession is driven by pioneer species preparing the way for later species on and into the climax.

Field Capacity: the water remaining in a soil after it has drained naturally.

Hartig Net - a net-like structure created by ectomycorrhizae fungal hyphae which envelops the cortical cells of the plant root and facilitates nutrient exchange between the plant and the fungal host (Sylvia et al. 2005)

Mutualism: a relationship between species that benefits both. Can be symbiotic or nonsymbiotic.

Mycorrhizae - a term used to describe a symbiotic association between root colonizing fungi and plants (Sylvia et al. 2005)

Niche: an organism's role, function, or position in an ecosystem.

Niche Differences: those that keep organisms from competing for resources

Rhizosphere - the zone of chemical, biological, and physical influence generated by root growth and activity. The concept usually pertains to the soil-root interface but is sometimes extrapolated to other media-root interfaces (Pinton et al. 2007)

CHAPTER 1 INTRODUCTION

1.1 Background and introduction to mutualisms

Ecology is the study of relationships of organisms to one another and to their physical environment. There has now been over 150 years of research into what Darwin named "the entangled bank" of species interaction and much still needs to be discovered about the complex ecological networks which exist (Darwin 1859). Mutualisms, beneficial partnerships between organisms or species, are ubiquitous in nature. Organisms and their mutualists form extensive webs of interactions, which are undeniable in their importance, but incredibly difficult to study and understand in their complexity (Jordano 1987b, Thuiller et al. 2013). These interactions direct ecosystem development and functioning, community assembly as well as determine the resilience and stability of ecosystems (May 1972b, McCann et al. 1998, Ives and Carpenter 2007).

Mutualisms are everywhere, and though seen as a typically weak biotic interaction when compared to predation or competition, they are essential to most terrestrial ecosystems, with an estimated 98-99% of plant species dependent on some type of partner (Bawa 1990). This significance of mutualisms can be seen in plant pollination and dispersal partnerships, nutrient exchanges, as well as obligatory associations during certain life phases of both plants and animals (Bronstein 1994, Bascompte and Jordano 2007). As environmental change draws mounting concern at a global scale, there is increased demand to understand ecological services and the impacts of global climate change on resource levels in the environment (Rillig et al. 2000, Tylianakis et al. 2008). The consequences of increasing temperature along with the impact of other abiotic factors on intricate biotic functioning remain difficult to incorporate into current theory and environmental modelling (Thuiller et al. 2013). Amelioration of environmental stress is crucial in maintaining our current use of resources and will help to improve the services that the entire planet needs to survive. Mutualisms help stabilise ecological networks through mutually positive interactions, and can amplify the resilience of an ecosystem when these relationships are intact. The presence of an advantageous partner can provide a buffer against harmful events and facilitate co-survival in otherwise unsuitable circumstances, or allow a species to expand into a formerly inaccessible range (White et al. 2006, Van der Heijden and Horton 2009, Afkhami et al. 2014). Plant invasions of new territory are of growing concern, and

knowing how a species range may change precisely will be vital in land management decisions (Richardson et al. 2000). Ecologists needs more data from the field to expand on their current understanding of the roles of species at the community level, and how these roles change with differing abiotic factors. Determining where mutualisms are the most beneficial and why will improve our knowledge of how to preserve these interactions for the future.

Predation is the biotic interaction that has received the most interest over ecological history (Sexton et al. 2009). Studying predation relationships is often straightforward, but not easy, to quantify the loss of one group and the gain of the other (e.g. number of prey killed), leading to very clear empirical understanding of predator-prey cycles (May 1972a). However, in mutualisms, the participants are often less visible, or only important under certain circumstances, making the situation less quantifiable. It is also important to identify that many mutualisms derived from originally antagonistic relationships. Thus each partner has evolutionarily adapted for their needs, and utilizes their partner as an energy resource or to increase efficiency. The degree of specialization, and hence obligation, in these relationships is often unknown across different ecosystems, leading to further complication (Jordano 1987a). In particular, the study of mutualisms has primarily been done on networks of plants and their respective insect pollinators and dispersal agents (Bronstein et al. 2006). While these are essential aspects of all terrestrial ecosystems, they are not the only mutualistic relationships present in nature. The variability of mutualisms has not yet been united by ecological theory, partially due to the extensive variety of the organisms that participate, and the wide range of services that are exchanged between parties and the isolation between the studies themselves (Bronstein 1994). The gap between theoretical and empirical studies is preventing a wider picture of mutualist interactions from being formed, especially when looking across trophic levels (Crowley and Cox 2011, Kissling and Schleuning 2015).

Much remains to be learnt about the complex networks of mutualistic interactions and how these linkages are affected by abiotic factors. This Master's project focuses the mutualisms that exist between the *K. ericoides* and its fungal partners, arbuscular mycorrhizae and ectomycorrhizae, and how these two interactions may be affected by changes in the amount of available moisture.

1.2 Mycorrhizae

Mycorrhizae are fungal organisms which are present in the soil and live in association with terrestrial plant roots. This relationship with plants can range from symbiotic to pathogenic, depending on the nutrient and energy transfers between the two partners (Smith and Read 2010). Three main types of mycorrhizae exist: arbuscular mycorrhizae fungi (phylum *Glomeromycota*) and ectomycorrhizae fungi (phylum *Basidiomycota*, *Ascomycota*, and few *Glomeromycota*), and ericoid mycorrhizae fungi (phylum *Ascomycota*) (Schüßler et al. 2001). As soil studies have improved, researchers have confirmed the widespread association between plants and soil fungi, and the dramatic and fundamental effects these interactions have on plant community structure and diversity (Allen 1991, Bever et al. 2010, Klironomos et al. 2011, Dickie et al. 2013). This project focuses on arbuscular mycorrhizae (AMF) and ectomycorrhizae ECM as these are the two fungal groups that associate with the study plant *K. ericoides.*

80% of all plant species, and 92% of all plant families form soil mycorrhizal mutualisms of some kind, and mycorrhizae are present worldwide from tropical environments to the poles (Allen 1991, Wang and Qiu 2006, Timling et al. 2014). These mutualisms allow for greater access to nutrients and water due to the higher surface area of fungal hyphae. Mycorrhizae, in fact, are responsible for most nutrient uptake for terrestrial plants (Smith and Read 2010). Often these mycorrhizae can absorb different mineral forms of nutrients, increasing the amount of resources available to the plant (Bever et al. 2010, Marschner and Marschner 2012). In return for this increase in nutrient availability, the mycorrhizae have regular access to proteins and sugars from plant roots, which are necessary for growth (Allen 1991). Mycorrhizae, with few exceptions, are completely reliant on their relationship with a plant for organic carbon, which permits them to highly competitive for phosphorus and nitrogen sources in the soil (Allen 1991, Smith and Read 2010).

Of those species that form mycorrhizal associations, 95% of them form associations with arbuscular mycorrhizae (Allen 1991). AMF interactions are especially common in ecosystems with high plant diversity, and from fossil records, were shown to evolve 1000-460 million years ago; this ancient relationship may allow AMF to colonise almost all land plants (Simon et al. 1993, Smith and Read 2010). Also, AMF mutualisms are thought to be the reason green plants were originally able to invade terrestrial habitats, as the earliest plants had no true root systems

(Malloch et al. 1980, Simon et al. 1993, Smith and Read 2010, Willis et al. 2013). All species of AMF are considered obligate mutualists and are completely dependent on autotrophic plants for an organic carbon source (Smith and Read 2010). AMF are not known for their diversity themselves, and it is fairly common for highly diverse above-ground vegetation to host only a few types of AMF in their rhizosphere (Read 1991).

AMF penetrate the cell walls of vascular plants and mostly exist intracellularly, with external hyphae allowing AMF to access soil pore space up to an order of magnitude smaller than plant roots (Smith and Read 2010). These external hyphae allow for increased soil exploitation for moisture and nutrients by AMF (Smith and Read 2010). And it has been shown through extensive research to have the largest effect on plant phosphorus (P) nutrition, as it has low mobility in soils and is required in relatively large amounts by both fungus and plant (Smith and Read 2010). Recently, AMF have been revealed to have a higher capability for nitrogen uptake and transport than ECM, but they cannot access the wider range of nitrogen compounds that are present in soil (Bever et al. 2010). It has also been suggested that AMF colonisation can increase plant fitness by augmenting disease resistance, resistance to insect herbivory, and increase drought tolerance (Smith and Read 2010).

The remaining 5% of mycorrhizal plant species (3% of all seed plants) form relationships with ectomycorrhizae, and almost all are woody perennials (Allen 1991, Smith and Read 2010). The oldest associations of this fungus date back to approximately 130 million years and are found in conjunction with family Pinaceae (LePage et al. 1997). In contrast to AMF, ECM associations are more common in habitats with lower plant diversity, or ecosystems with a dominate species (Smith and Read 2010). Ectomycorrhizal fungi are very diverse both structurally and functionally, with 5000-6000 fungal species estimated to form mutualisms, making universal features of this group difficult to define (Smith and Read 2010). Many dominant forest-tree families form ectomycorrhizal associations; including important timber species such as Pinaceae (Pine trees, e.g. *Pinus radiata*, *Pinus negra*) and Myrtaceae (Eucalypts e.g. *Eucalyptus globulus*, *Eucalyptus sideroxylon*), temperate forest dominants such as Fagaceae (Oak trees e.g. *Quercus alba*, *Quercus robur*), and Betulaceae (Alder and Beech trees, e.g. *Alnus rubra*) (Smith and Read 2010). And significant to New Zealand ecology, the southern beech trees, from the genus *Nothofagus* are also an ectomycorrhizal obligate in when growing in nature (Read 1991). Thus, it is apparent that a mutualistic relationship with ECM, rather than AMF, may contribute to the

relative importance of a plant species in an ecosystem, allowing it to play a greater role in ecosystem structure than expected.

ECM form a mantle or fungal sheath which surrounds the root with hyphae radiating both outward into the soil (these external mycelia may be seasonal) and inward forming the Hartig net (Smith and Read 2010). The Hartig net hyphae exist in the spaces between the plant cells, and even though intracellular penetration is possible by most ECM species, it considered uncommon and weakly pathogenic when it occurs (Smith and Read 2010). ECM mutualisms are most developed in ecosystems limited by nitrogen levels, such as northern hemisphere temperate and boreal forests (Smith and Read 2010). The high species diversity of ECM is reflected in the wide range of extracellular proteinase enzymes produced, with most species able to access different nitrogen compounds including nitrate, ammonium, and some simple organic-N compounds (Smith and Read 2010). These compounds are not available for arbuscular mycorrhizae transport, thus giving an advantage to ECM plant species growing soils with low levels of nitrogen (Read and Perez-Moreno 2003, Bever et al. 2010). In terms of phosphorus transport, ECM species can produce phosphatases to breakdown sources of organic phosphorus, reaching similar levels of transport as AMF systems (Smith and Read 2010). Also, with the presence of large external networks of mycelium, ECM have been found to be capable of interplant transfer of nutrients and also increase tolerance of heavy metals and of drought conditions (Smith and Read 2010). These qualities of ECM support the idea that these relationships are more proactive in nutrient transport and acquisition when compared to AMF mutualisms.

Dual-mycorrhizal plants are can be found in several shrub species and tree families Salicaceae (willow, poplar and aspen) (Lodge and Wentworth 1990, Van der Heijden and Vosatka 2000) and Myrtaceae (Lapeyrie and Chilvers 1985, Jones et al. 1998), which includes the study species for this research *K. ericoides*. In cases of one plant hosting both types of mycorrhizae, ECM has been found to been the dominant coloniser (Smith and Read 2010). However, when AMF and ECM plant species are growing in conjunction, ECM presence is diminished due to possible competition for nutrients (Smith and Read 2010). It has been suggested that the age of the host plant may affect which mycorrhizal type is present as AMF may be succeeded by ECM as plant moves from establishment phase to adulthood (Lapeyrie and Chilvers 1985, Moyersoen and Fitter 1999). Also, as AMF associations arose millions of years before ECM existed and were

present in nearly all terrestrial plants, the ability of ECM plants to host AMF may be a residual evolutionary trait (Vardavakis 1992, Cázares and Trappe 1993).

Many plants have become dependent on the service of these soil fungi to deliver nutrients, especially in the establishment phase of the plant life cycle (Moyersoen and Fitter 1999). It is possible that under different sets of environmental conditions, the reciprocal exploitation of the two partners may become more or less beneficial. Increased specialization or obligation of the mutualism may affect the efficiency of the exchanged services, perhaps limiting the locations where each of partners may survive. Understanding how abiotic factors can affect the level of benefit will help determine response of a species to the ongoing changes in global climate.

1.3 Historical ecology of New Zealand

It is well known that much of the native flora and fauna of New Zealand is unique due to its 80 million years of geographic isolation, which has included several land transformations and widespread inundations (Moyersoen et al. 2003). The distinctive environmental qualities provide new scenarios in which to explore ecological theory under the consideration of island dynamics.

The arrival of humans changed the ecological processes of the islands through hunting, use of burning regimes for agriculture, and most dramatically the introduction of invasive species; especially mammalian introductions (Ewers et al. 2006, McWethy et al. 2010). As alien species arrived and agricultural practices have intensified, many native species were driven to extinction or threatened status (Blackwell et al. 2008). This drastic change in ecological forces has lead to emphasis in scientific study on control of invasive species, habitat restoration, and maintenance of ecological services.

Historically, New Zealand was mostly covered by forest and shrubland, both environments include *Kunzea ericoides* as an important successional species (Wardle 1991). While Kanuka is still widespread in New Zealand, it has a declining distribution mostly due to removal by farmers who see it as a pest in recently cleared pastures, and overall expansion of agricultural land use (Blackwell et al. 2008). The relatively recent rise of society's concern for environmental protection has left New Zealand fighting to save its native flora and fauna from alien invaders

and trying to re-establish native habitats. Although many places are unlikely to return to their pre-human state, the study of the remaining original landscape can improve land management design which in turn will improve ecosystem services. Many native plant species, especially woody species like Kanuka, have increasingly restricted distributions, but are essential community participants in the regeneration of natural ecosystem and nutrient cycles (Wiser et al. 2011). Understanding how Kanuka and other native species establish new populations in the environment and their role in forest succession will allow higher quality restoration projects to be developed. More informed restoration actions will help to improve current land management practices by increasing sustainability of species, ecological services, and help in maintaining long-term environmental projects.

1.4 Biology and ecology of the *K. ericoides* complex

1.4.1 Taxonomy and morphology

Kunzea ericoides var. ericoides (manuoea, manuka-rauriki, white tea-tree, kanuka) is the most recognized form of kanuka in New Zealand. It is in the Myrtaceae family which includes 133 genera and more than 3800 species (Stephens et al. 2005, McKenzie et al. 2006). Manuka and kanuka were assigned to the same genus (*Leptospermum*) until the early 1970s when kanuka was moved to the *Kunzea* genus, which contains 65 species, which are mostly of Australian origin (Metcalf 2011). Until 2014, there were only three recognised New Zealand endemic *Kunzea* species, with up to four more species being considered for further taxonomic clarification (Metcalf 2011). The three species were: (1) The widespread and fairly common *Kunzea ericoides,* (2) *Kunzea ericoides var. microflora*, a species which occurs in geothermally active habitats, known as prostrate kanuka; and (3) *Kunzea sinclairii*, a kanuka species that is restricted to the Great Barrier Island (Metcalf 2011). In 2014, research continued to refine the genetic and distributional differences between plant types, proposing that New Zealand is actually home to 11 species of kanuka, of which seven are newly described, and most are proposed to be natural hybrids (de Lange 2014). While recognising the differences between the variable habitats of kanuka types has increased the amount of available information, it is arguable whether the differences demand such specific classification when considering the physical and especially ecological similarities across the newly defined species list. However, it has long been

recognized that kanuka is widely variable across New Zealand, but the large-scale genetic, cytologic, geographic patterns have been difficult to track and confirm and the ecological differences still need to be examined further (de Lange 2014). This literature review focuses on *K. ericoides*, which a species endemic to the South Island of New Zealand, and was the species used for this research project. In this thesis, *K. ericoides* refers specifically to this plant, which kanuka refers to all considered species.

1.4.2 Description of *Kunzea ericoides*

K. ericoides is an evergreen tree can live as long as 160 years and grow up to 18 meters tall by six meters wide, with slender branches (Wardle 1991, Metcalf 2011, Carswell et al. 2012, de Lange 2014). It grows one to four flexible trunks, with the lower portion (1 – 4 m) devoid of branching (de Lange 2014). The mature trunk circumference is 10 to 60 cm with papery light brown-grey bark which sloughs off in long strips (Wardle 1991, Metcalf 2011, de Lange 2014). It has narrow, lanceolate leaves approximately 1 cm in length and 2-3 mm in width, which are pointed but not sharp (Wardle 1991). Overall, the *K.ericoides* tree can vary widely in leaf colour, but is usually has a bright yellow-green or olive-green coloration when viewed from far away, with most leaves occurring at the very ends or top of the branches (de Lange 2014). Its wood is reddish in colour and has a relatively high density (Wardle 1991). It produces white 5-petaled flowers (approximately 6 mm in diameter), which occur in bunches, from November to March (de Lange 2014). The fruit becomes ripe in April and May (Burrows 1996, Metcalf 2011). The fruit is barrel-shaped, and does not persist on the branch (de Lange 2014). Their tiny, black seeds are wind dispersed from small 0.5 cm capsules, contributing to its ability as a pioneer species after disturbance events (Sullivan et al. 2007).

1.4.3 Distribution and Habitat

Genus *Kunzea* occurs in Australia and throughout the islands of New Zealand. Native shrubland currently covers 10% of New Zealand's 27 million hectare landmass and suffers from the continued impacts of invasive species, fire, and land-use changes (Thompson et al. 2004). Kanuka species typically occur in conjunction with manuka (*Leptospermum scoparium)* and these mixed stands make up 5.2% of the native shrubland cover (Whitehead et al. 2004). One of the major threats to kanuka and manuka scrubland is gorse (*Ulex europaeus*), an aggressive,

introduced legume that is becoming the dominant species following disturbance events (Magesan et al. 2012). Kanuka and manuka stands historically covered wide swaths of the South Island, but fell into decline with the rise of fertilizer-dependent farming, which dramatically increased in the 1940s (Wardle 1991). In some parts of Australia, kanuka species are considered invasive (Singer and Burgman 1999).

Throughout its range, kanuka trees are extremely drought tolerant and continue successful growth during severe drought due to adaptations for survival with low internal water potentials (Innes and Kelly 1992). Kanuka species often form dense stands and establishes quickly after disturbance, but can also occur in forest openings or along forest edges with sufficient light exposure (Burrows 1996, de Lange 2014). Several species are fairly common throughout Canterbury (*Kunzea ericoides*, *Kunzea serotina*) and abundant on Banks Peninsula (*Kunzea robusta*) (Wilson and Galloway 1993, de Lange 2014). Across the South Island, riverine flats with fine-deposit bottoms, as opposed to rocky, can be dominated by kanuka if left undisturbed by flooding (Wardle 1991, Gibb 1994). On the North Island, kanuka is an important species near geothermally active areas. *Kunzea tenuicaulis*, previously known *Kunzea ericoides var. microflora*, as is one of the few species that can inhabit the high temperature soils near volcanic sites in its prostrate morph and is a distinct part of the predictable floral zonation of this ecosystem (Boothroyd 2009, de Lange 2014).

Kunzea ericoides is preferentially found in free-draining, rocky soil and is less vigorous and unlikely to grow in waterlogged soils, and occurs from coastal to montane shrubland, scrub, and low forest (Wilson and Galloway 1993, Wiser et al. 2011). This species is a primary coloniser of formerly forested habitat on a variety of soil types including: sand, loam, clay, alluvium, igneous, sedimentary and ultra-mafic rock (de Lange 2014). It is able to outcompete manuka in fertile, dry soils and most commonly occurs from sea level up to approximately 500 m, but can exist at elevations up to 1600 m of elevation (Metcalf 2011, de Lange 2014). *K. ericoides* usually inhabits the drier areas of New Zealand, which can be occupied by woody plant species; and in such habitats this shrubland is self-perpetuating rather than successional to broad-leaved species (Wardle 2001, Wiser et al. 2011). It is common throughout the northern half of the South Island, on which it is endemic, and it is not listed as threatened (de Lange 2014). In Westland, *K. ericoides* is confined to well-drained river plains; it does not often develop past Hokitika to the south nor to the west of Clutha Valley (Wardle 1991, Burrows 1996).

1.4.4 Successional importance

Establishment and enhancement of woody ecosystems presents a vast opportunity in New Zealand to increase native biodiversity and ecosystem resilience. Successional progression of inhabitation of an ecosystem is a founding idea of ecology (Clements 1936) and study of this type of ecosystem development in New Zealand is crucial to future restoration projects (Blackwell et al. 2008). Kanuka species are well known to provide sheltered habitat for the slower growing tree species of New Zealand such as kohekohe (*Dysoxylum spectabile*), tawa (*Beilschmiedia tawa*), and kauri (*Agathis australis*) (Wilson and Galloway 1993, Atkinson 2004, Wiser et al. 2011). The role differs from the ecologically and morphologically similar manuka (*L. scoparium*) shrub as kanuka is much longer living and grows to a larger size. Kanuka can be a pioneer species, but is usually part of the second stage of regeneration as a relatively fast growing woody plant (Allen et al. 1992). The turnover from dense kanuka stand to broadleaf forest is slow, as after kanuka establishes a closed canopy, there is a very low rate of change in species composition as self-thinning must occur before other species can encroach on the occupied space (Wardle 1991, Allen et al. 1992). Once the stand is approximately 50 years old, the inability of kanuka to expand its canopy to fill tree fall gaps and lack of self-regeneration under its own cover, allows more shade tolerant slow-growing trees to establish in the understory, and *K. ericoides* will be mostly replaced by 150 years (Allen et al. 1992, Burrows 1996, Atkinson 2004). It is thought that the open structure of mature kanuka stands may catch seeds of other wind and bird pollinated species and then provide sufficient light for successful seedling establishment (Ecroyd and Brockerhoff 2005). It has been shown that kanuka/manuka stands have led to broad-leaf assemblages that are more authentic in their species assemblages than reforestation which occurs under exotic vegetation, such as gorse (*Ulex europaeus*) or radiata pine (*Pinus radiata*) (Sullivan et al. 2007, Brockerhoff et al. 2008). Although kanuka/manuka stands are transient, natural disturbance regimes maintain their presence under normal circumstances. Land managers may be able utilize the facultative ability of native shrubs to increase native forest cover and native species diversity (Sullivan et al. 2007).

1.4.5 Facilitative capabilities

The environment created by kanuka shrubland has allowed for high levels of indigenous diversity to exist in the small remaining fragments of native bush in the Canterbury plains and elsewhere (Ecroyd and Brockerhoff 2005). It provides habitat for several rare, native-forest specialized and critically endangered ground beetles of the family *Carabidae* (Harris et al. 2004, Brockerhoff et al. 2005, Berndt et al. 2008). Kanuka and manuka stands can also persist on waste rock leftovers from open cast mining. They can colonise steep slopes of up to 40 degrees without human intervention and in this habitat outcompete gorse, thereby creating native habitat space for the recovery of invertebrate species (Rufaut et al. 2006). Also, kanuka has been known to re-invade exotic pine forests. This demonstrates that conversion of pine forests back to native New Zealand broadleaf is possible even without direct human intervention (Ecroyd and Brockerhoff 2005). The capability of kanuka to establish in adverse conditions supports its use as a resilient ecosystem restorer.

1.5 Ecological services provided by *Kunzea* species

1.5.1 Tea Tree Oil

Many of the studies of kanuka trees have focused on tree products, especially tea tree oil. It is known to be one of the species that produces the oil and this oil is similar in composition to the other oil producing species originating in Australasia (Lis-Balchin and Hart 1998, Lis-Balchin et al. 2000, Wyatt et al. 2005, Maddocks-Jennings et al. 2009). While formal kanuka farms have not been established, the large commercial market for this product (Davis 2003) has made tea tree oil the focus of research rather than ecological issues.

1.5.2 Carbon Sequestration

There has been increased interest in the use of fast-growing woody species as carbon stores to slow down the effects of climate change and reduce the heightened levels of carbon dioxide in the atmosphere. Due to its role in the successional progression of native forest and its fast-growing life history, kanuka and manuka stands are able to utilize and store a high volume of carbon each year (Carswell et al. 2012). The mean amount of carbon sequestration by kanuka

stands under 50 years old was recorded as up to 61.5 ± 7.7 Mg of carbon per hectare; which is similar to the amount of carbon stored by *Pinus radiata* plantations in their first 20 years after planting (Scott et al. 2000, Carswell et al. 2012). Even after self-thinning, which occurs after the stand reaches approximately 50 years old, the amount of above ground biomass does not drop until the transition to broadleaf species occurs (Pearce et al. 2010, Carswell et al. 2012). Also, soils beneath kanuka act as a sink for methane, decreasing oxidation rates, and thus lowering overall movement of carbon into the atmosphere, during the conversion from pasture to shrubland (Price et al. 2010). The successional pathway followed by kanuka reaches high levels of native plant diversity (Ecroyd and Brockerhoff 2005), and increased vegetative complexity has been shown sequester larger amounts of carbon that monotypic or low diversity habitats (Cannell 1999, Paquette et al. 2009, Strassburg et al. 2010, Schleuss et al. 2014). The kanuka tree could be useful tool available to the environmental protection agencies of New Zealand when considering how to both lower carbon emissions at a large scale while creating new native habitat (Whitehead et al. 2004). For example, in order to comply with the new international environmental regulations, the New Zealand government can use a plan to reforest marginal agricultural or pastoral land with kanuka shrubland to produce a significant offset of greenhouse gas emissions (Scott et al. 2000, Trotter et al. 2005, Price et al. 2010). When considering current climate change compensation, the ability of kanuka scrub to establish in adverse habitat and quickly grow shows it can provide anthropogenic service beyond native habitat restoration.

1.6 The Dual-Mycorrhizal associations *Kunzea ericoides*

K. ericoides, the study species for this project, is unusual in that it forms a mutualistic relationship with both AM and ECM (Moyersoen and Fitter 1999, McKenzie et al. 2006, Weijtmans et al. 2007). Usually, these two fungal groups form exclusive pairs with their hosts: plants either have associations with AMF or ECM, but not both (Moyersoen and Fitter 1999). There are approximately 40 ECM species that form associations with kanuka (Orlovich and Cairney 2004). Only three native woody genera in New Zealand form associations with ECM fungi in any capacity: *Kunzea* (kanuka), *Leptospermum* (manuka), and *Nothofagus spp.* (beech) (Orlovich and Cairney 2004). It has been proposed that this ECM mutualism contributes to the ability of kanuka and manuka to facilitate establishment of beech species (Moyersoen and Fitter

1999, Weijtmans et al. 2007). Even though beech species are dominant in approximately 70% of New Zealand forest, is has been suggested that the spread of *Nothofagus* into grassland is extremely slow due to lack of appropriate mycorrhizal inoculum for the seedlings (Dickie et al. 2012). By hosting ECM, Kanuka not only provides the above ground habitat for successful establishment, but can support the rhizospheric needs of both AMF and ECM species. This facultative ability may also increase in areas of high abiotic stress where the benefits of fungal-seedling associations outweigh the competitive aspects between trees and pioneer species (Grau et al. 2010). Because pine trees are also ectomycorrhizal and exotic to New Zealand, this may contribute to the ability of both plants to invade mature stands of one another (Orlovich and Cairney 2004, Davis et al. 2011, Dickie et al. 2011). The ability of kanuka to associate with both types of mycorrhizae provides an interesting system to study how mutualisms are affected by abiotic and biotic factors

1.7 Aims and Objectives

The overall goal of this project is to explore the relationship between *Kunzea ericoides* and its two mycorrhizal mutualists, and investigate how this association is affected by available moisture levels across New Zealand's South Island. The experiment is split into two distinct phases: a field sampling of kanuka across the South Island and a controlled glasshouse experiment growing *Kunzea ericoides* from seed.

The first set of objectives focuses on where each mutualism occurs, focusing on the effect of rainfall on the presence of each ECM and AMF. The goal is to show how mycorrhizal mutualisms can be affected by abiotic gradients on a large scale in order help recognize the geographical nature of mutualistic interactions. The main goals are to: (1) confirm the ability of *Kunzea ericoides* to form a mutualism with both arbuscular mycorrhizae and ectomycorrhizae, (2) measure the effects of within site micro-topography on the levels of fungal colonisation, and (3) measure the level of AMF and ECM colonisation across the rainfall gradient of the South Island. These will test the hypothesis that arbuscular mycorrhizae are the dominant fungal mutualists with *K. ericoides* in areas of high rainfall and ectomycorrhizae are the dominant fungal mutualists with *K. ericoides* in areas of low rainfall.

The second part of the research focuses on assessing the effect of ectomycorrhizae on the growth of *Kunzea ericoides* seedlings when experiencing various levels of water stress. This will help to understand why ECM may be more effective in certain environments and demonstrate how the ability to form relationships with both ECM and AMF allows *K. ericoides* to have a larger than expected environmental range. The main objectives are to: (1) measure the level of ECM colonisation of *K. ericoides* under varying moisture conditions and (2) measure growth rates of *K. ericoides* seedlings under a varying watering schedule. These measurements will help test the hypothesis that ectomycorrhizae colonisation increases the growth rate of *Kunzea ericoides* under drought conditions and the hypothesis that ectomycorrhizal inoculation will alleviate water stress when *K. ericoides* is grown under drought conditions.

Chapter 2 Field Survey

This chapter describes the materials and methods used for the field section of this research and the results and discussion from the field work section of this thesis. There will be an overview of the materials and equipment used as well as basic theory when needed. By combining the field work with the glasshouse experiments (see Chapter 3), a more in depth measurement of mycorrhizal mutualisms and their effect on the growth rate of kanuka (*Kunzea ericoides*) over a hydrologic gradient is possible (Morales et al. 2014). Specifically, we want to: (1) Confirm the dual-mycorrhizal nature of *K. ericoides*, (2) measure the effects of micro-topography with each site on levels of fungal colonization. (3) Determine if high rainfall levels increase the strength of association between *K. ericoides* and AMF.

2.1 Site Selection and Background

The site selection for seedling acquisition was important to this study as empirical data for the relationship between kanuka species and mycorrhizae is lacking. The data provided by the site samples will be used to create a basic map of changing mutualistic relationships over an environmental gradient. The samples were taken over the natural rainfall gradient that exists laterally across the South Island of New Zealand. Due to the placement of the Southern Alps, the West Coast can receive on average 400 to 800 cm of rain annually, while Canterbury on the east coast receives only 50-75 cm as recorded by the National Institute of Water and Atmospheric Research (NIWA) (see Fig 2.1).

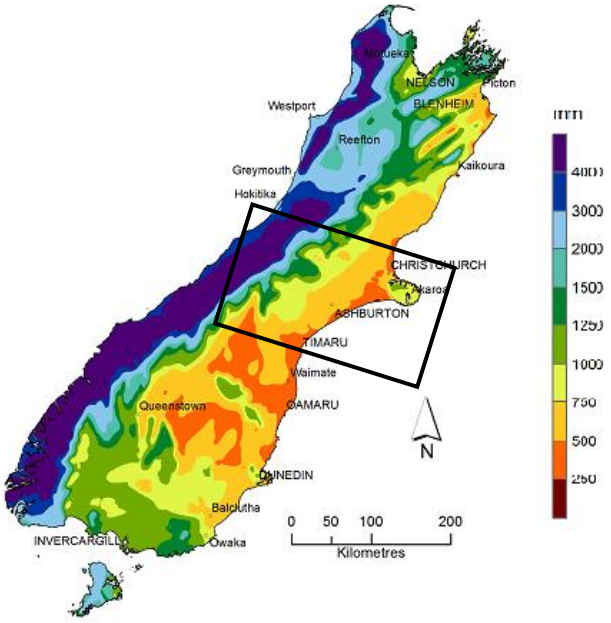

Figure 2.1. Average rainfall (mm) across the South Island of New Zealand (1971-2000) as recorded by NIWA (shown with permission). Sampling area is highlighted by the black rectangle.

The most important aspects of the considered field sites were: presence of study species and known recorded levels of rainfall. The secondary consideration was ease of access in both legal and physical terms. The location of each field site was recorded using a handheld Global Positioning System (GPS) and mapped onto digital satellite photograph of the South Island (see Fig. 2.2).

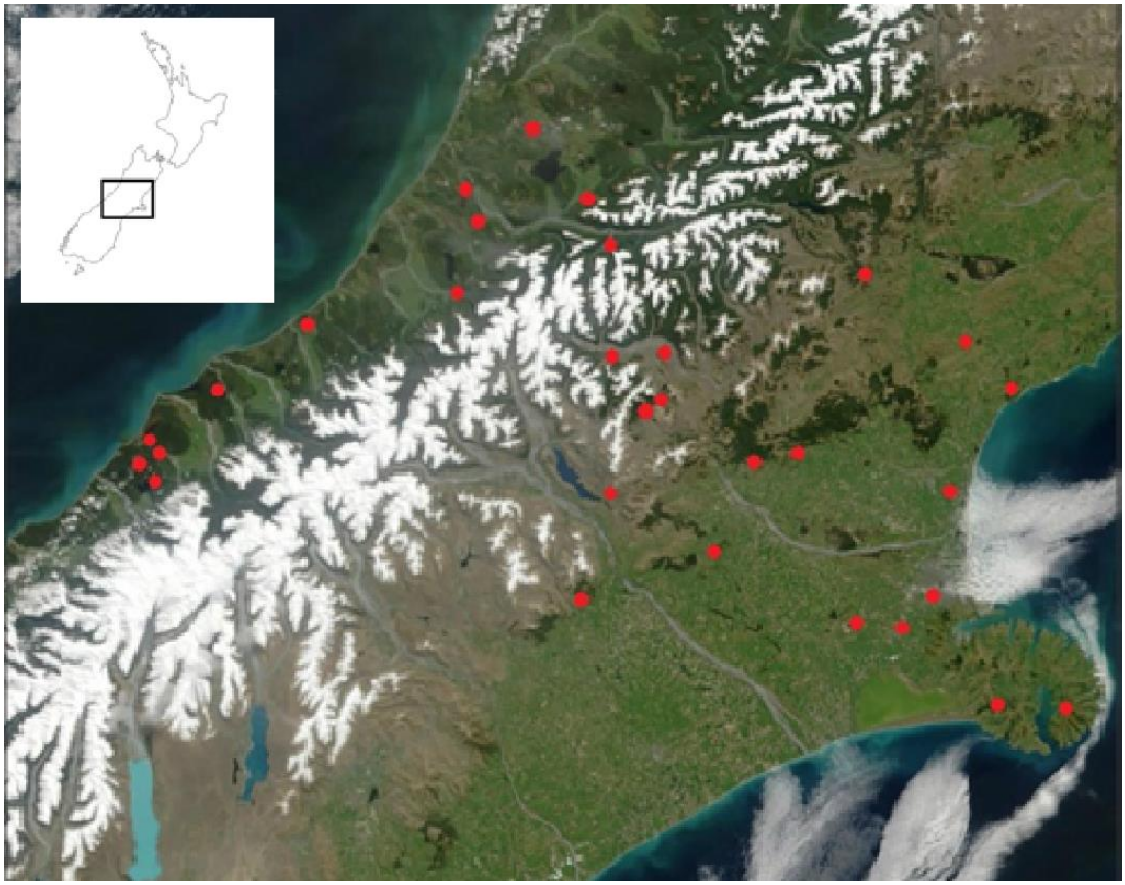

Figure 2.2. This image shows the 30 sites used to sample *K. ericoides* used for this project. Photo courtesy of the NASA Visible Earth.

The field sites for collecting of *K. ericoides* seedlings ranged across the south island of New Zealand, through Arthur's Pass. There were 30 sites used for seedling collection (See Fig. 2.2, Table 2.1) ranging from coastal to alpine sites. All samples were gathered during the 2013/2014 summer season. The rainfall records were acquired from annual data taken by NIWA. A description of vegetation coverage and type was done for each site as well as several

photographs taken. Seedlings were identified down to the species level in the field when possible, or later confirmed in the lab when the exact *Kunzea* species was uncertain. The three seedlings were selected per site based upon the micro-topography, with one seedling taken from each a convex, concave, and flat area where available. These different samples helped to determine if there was any relationship between each type of topography and mycorrhizal colonization, based upon differences in the microhabitat within each site, as has been suggested (Posada et al. 2008). A larger overall sample size was also acquired by collected a total of three plants per location. Each seedling was taken with a small amount of surrounding soil. This preserved the entire root structure of each plant and protected the fine root tips, which are the main host roots for mycorrhizae. The slope, altitude, and approximate percentage cover of kanuka/manuka were also recorded for each site. Any of these factors may have some correlation or causation on the fungal infection or mutualism functioning for either ECM or AMF.

Table 2.1. Site information for field sampling of *K. ericoides*.

Site Name/ Location	Latitude	Longitude	Rainfall (mm)	Kan/man Cover (%)	Altitude (m)	Slope (degrees)
Christchurch	-43.532303	172.593375	500	0	9.79	0
Burnham	-43.605573	172.31472	600	5	65.34	0
Lincoln	-43.639881	172.478051	600	40	11.81	0
Weka Pass Rd	-43.002532	172.708563	600	5	179.17	10
Tiromoana Lookout	-43.094047	172.849773	600	95	241.62	15
Lake Coleridge	-43.342591	171.603389	800	20	574.48	15
View Hill	-43.266227	172.076689	800	5	355.82	10
Glentunnel	-43.461985	171.915875	900	5	358.37	5
Banks P1 - Vista	-43.809243	172.788045	900	20	394.04	10
Banks P2 – Summit	-43.747294	172.99376	900	15	448.23	10
Ashley Gorge Bdge	-43.229060	172.229485	1000	50	218.89	5
Pudding Hill	-43.565503	171.511451	1200	10	655.01	15
Craigieburn	-43.164135	171.719485	1200	20	795.94	5
Corner Knob	-43.14348	171.746747	1200	15	760.06	20
CASS – Sugarloaf	-43.030068	171.788401	1200	35	1018.04	15

Lake Sumner Road	-42.850608	172.394645	1200	30	489.01	20
Bealey Spur Track	-43.027684	171.62764	2000	65	625.19	25

Table 2.1 continued

Site Name/ Location	Latitude	Longitude	Rainfall (mm)	Kan/man Cover (%)	Altitude (m)	Slope (degrees)
Okarito 1 – Pakihi	-43.248943	170.205379	3000	65	75.57	0
Okarito 2 – Forks	-43.230349	170.247221	3000	75	52.27	10
Lake Wahapo	-43.248334	170.234647	3000	30	55.56	20
Hercules Reserve	-43.12149	170.431734	3000	0	38.94	0
Kakapotahi Rsve	-42.960266	170.700828	3000	40	24.85	0
Dillmanstown	-42.661906	171.205576	3000	80	132.65	0
Franz Joseph	-43.288835	170.229105	4000	5	96.38	0
Old Chch Road 1	-43.007211	171.744547	4000	65	173.84	5
Old Chch Road 2	-43.230349	171.207402	4000	75	180.27	5
Arnold Valley/Maori Creek	-42.542162	171.412388	4000	10	172.96	5
Mt. Alexander Rte	-42.688086	171.543028	4000	25	166.81	10
Lake Kaniere -Fox Rd	-42.892268	171.163993	5000	75	299.26	15
Deception Valley	-42.793525	171.62764	6000	35	298	5

2.2 Mycorrhizal Identification

For this research project, visual identification of fungal features by microscope was used to determine the presence or absence of both AMF and ECM on the roots of *K. ericoides*.

AMF colonization was confirmed by identifying unique fungal structures occurring internally to the plant's root which include: arbuscules, storage vesicles, and hyphae (Smith and Read 2010). Arbuscules, which are diagnostic of AMF colonization, are highly branched hyphae that form inside the cortical cells of the host plant (Abbott 1982, Clapp et al. 1995, Merryweather and Fitter 1998a). Vesicles often appear in near arbuscule structures and may or may not appear to be linked by hyphae (Smith and Read 2010). Intracellular hyphal coils may be present with or without arbuscules, but alone are not considered verification of AMF colonization (Dickson 2004). AMF also form external hyphae, but not at the same level of density or complexity as

ECM, and chlamydosphores may present during the fruiting period (See Fig. 2.3) (Smith and Read 2010). Staining of plant roots is often the best way to determine the presence of mycorrhizae, but it is especially essential for AMF as dying allows the internal fungal structures to be visible through the cellular walls of the plant (Abbott 1982, Clapp et al. 1995).

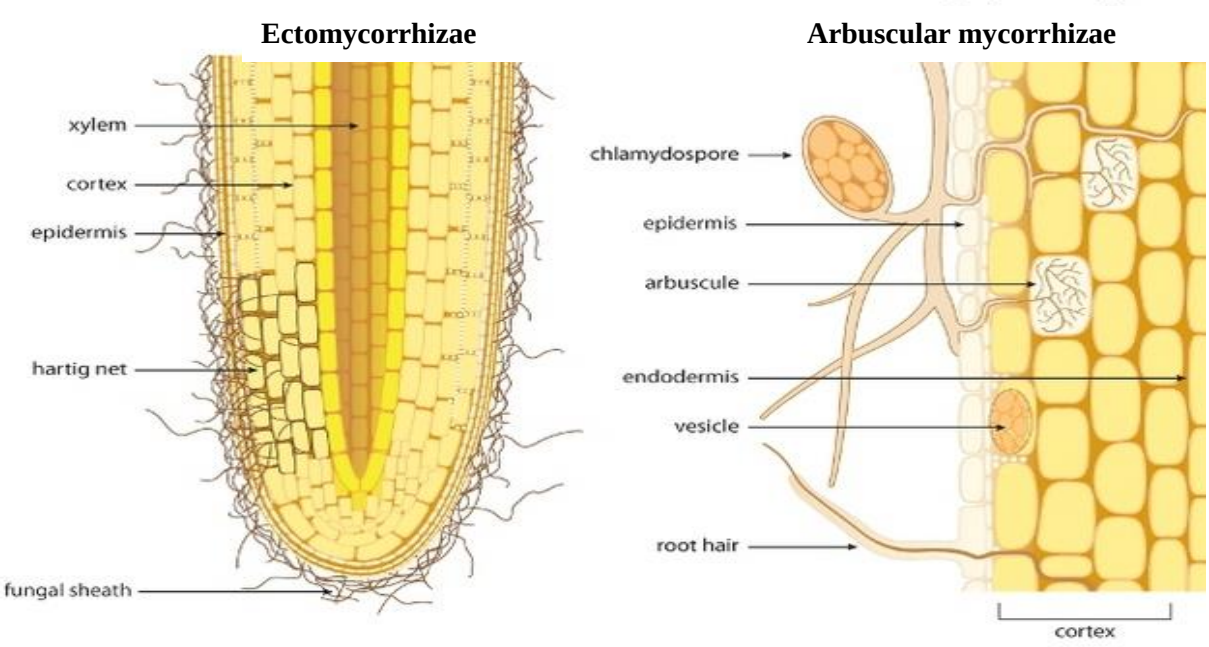

Figure 2.3. Ectomycorrhizae and arbuscular mycorrhizae each have unique features visible under magnification that diagnose their colonisation of root systems. Identifying these features was essential to knowing which type of mycorrhiza was present on *K. ericoides*. Image copyright - Nature Education (2013 Bonfante, P. & Genre, A. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nature communications* **1** doi:10.1038/ncomms1046).

Three main components characterize the presence of ECM: A mantle of fungal tissue which surrounds the root tip (hyphal sheath), an inward growth of hyphae between plant cells (Hartig net), and hyphae extending outwards from the fungal mantle (external mycelium) (Ingleby et al. 1990, Goodman et al. 1998, Agerer 1999, Agerer 2006). As the hyphal sheath and external mycelium may be visible to the naked eye or under low magnification, ECM is more straightforward to identify. This visibility can make staining unnecessary, but the high colouration of the stain can make quantifying colonisation amounts more simple (Ingleby et al. 1990, Weijtmans et al. 2007). When a root colonised by ECM is stained, the colouring of the hyphal sheath will obscure any internal structures, which could lead to a lower visibility for AMF.

These two techniques can be safely combined if the plant being examined is dual-mycorrhizal and was done in this research. The literature on mycorrhizal presence in New Zealand is not complete, and the variable character of these associations demands exploration at the local level to clarify the conditions which exist. There is room for further development of root management in the lab as different species demand varying amounts of processing for optimal mycorrhizal identification.

2.3 Analysis

The *K. ericoides* plants collected from the field had their roots cleaned of soil with water and then examined under a light microscope for presence of ectomycorrhizae, using a modified gridline intersection method (Phillips and Hayman 1970, Downs and Radford 2005, Dickie et al. 2012). This is where a grid is placed beneath the container holding the roots, and where a root visually intersects with one of the gridlines the root either recorded as "ECM", "AMF" or "No fungus" depending on the visual detection. As the seedlings collected for this research often had small roots systems, all of the roots were counted. This created an accurate record of total fungal colonization of the roots rather than estimate based upon a partial root count. The counting observations were done within two days after removal from the field. The roots were then cut from the above ground biomass, with the stems and leaves being kept for identification, and stored in 70% ethanol, which can be used to store roots for up to one year, until the root staining procedure could take place (Dickie et al. 2012). The roots were cleared of internal cellular structures using a modified 10% potassium hydroxide (KOH) stripping protocol adapted for the woody roots of the *Kunzea* genus (Brundrett 1991). The clearing of the roots was particularly challenging, requiring 68 minutes through a bench top autoclave for the necessary level of internal plant structure removal from the root. The staining procedure followed a standard protocol using HCl to acidify the roots before staining using Trypan Blue (Brundrett 1991, Dickie et al. 2012). The stained roots were then examined under 40-100x magnification to confirm ectomycorrhizal infection, specifically hyphal sheath and external mycelia presence (See Fig. 2.3-2.4); and to identify arbuscular mycorrhizal structures in the interior of the root, specifically presence of arbuscules, vesicle cells, and possibly chlamydosphores (See Fig. 2.3-2.4) (Brundrett et al. 1996). A total root count, the mycorrhizal type, and percentage of fungal

colonization were recorded for each of the samples based on a modified gridline method for estimating mycorrhizal presence (Newman 1966).

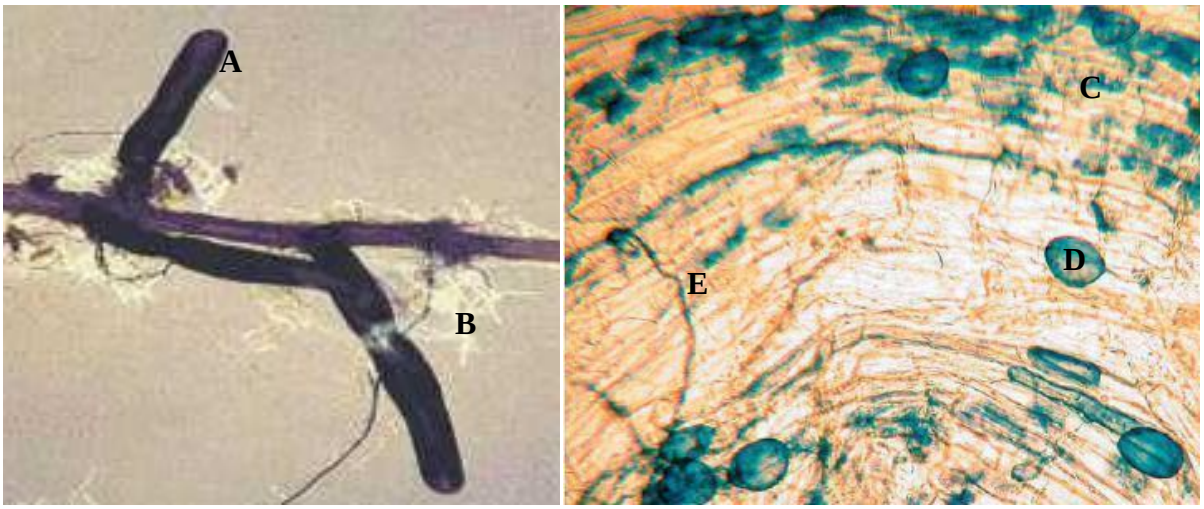

Figure 2.4. These pictures of *K. ericoides* show which features are used to identify both ectomycorrhizae (ECM) and arbuscular mycorrhizae (AMF). ECM is identified by the heavily dyed (A) mantle/fungal sheath, (B) external mycelia, and Hartig net (not pictured, see Fig. 2). AMF is identified by the presence of (C) arbuscules, (D) storage vesicles, and (E) hyphae.

2.3.1 Statistical Analysis

The effects of micro-topography on the different mycorrhizal colonization were examined using a one-way analysis of variance (ANOVA) test in R. Correlation and statistical significance between rainfall, kanuka/manuka coverage, altitude, and mycorrhizal colonization were also calculated. These tests together show if there is any significant relationship between the measured site variables and the presence of either type of mycorrhizae on the roots of *K. ericoides*.

2.4 Results

2.4.1 *K. ericoides* across the South Alps

From the 30 field sites, a total of 86 Kanuka seedlings were removed by hand for examination, as several sites did not have the different micro-topographical regions available (See Table 2.1). On the West Coast, *K. ericoides* was extremely difficult to locate, and often the sites had very

few specimens mixed in with many other plant species (See Fig. 2.5A). Coexistence with manuka was also extremely common, especially in waterlogged, coastal areas. In these circumstances, the well-accepted differences between manuka and kanuka were less (Wilson and Galloway 1993), defined and 11 of the samples needed identification confirmation in the laboratory. The dense forest on the West Coast prevents kanuka from being a dominant canopy species in most cases, instead growing along the forest edges where the light and moisture conditions suit its physiology. The mature *K. ericoides* stands that existed appeared further inland along braided river beds or along roadsides. In the more mountainous regions, manuka and kanuka were both present, but did not integrate as much as on the West Coast, mainly occurring in separate stands. The *K. ericoides* here often coexisted with other alpine shrubs, forming dense brush with *Hebe* and *Coprosma* shrub species. On the dry east coast, *K. ericoides* was much more abundant and easily identifiable. It often appeared in naturally dense thickets (See Fig 2.5B) and was even found growing in urban areas. *K. ericoides* grew tallest on the east coast, existing as the dominant canopy species in the mature stands with minimal amounts of plant growth in the deeply shaded understory. Overall, the distribution shows that kanuka generally thrives in dry conditions, and experiences higher competition in areas of high rainfall.

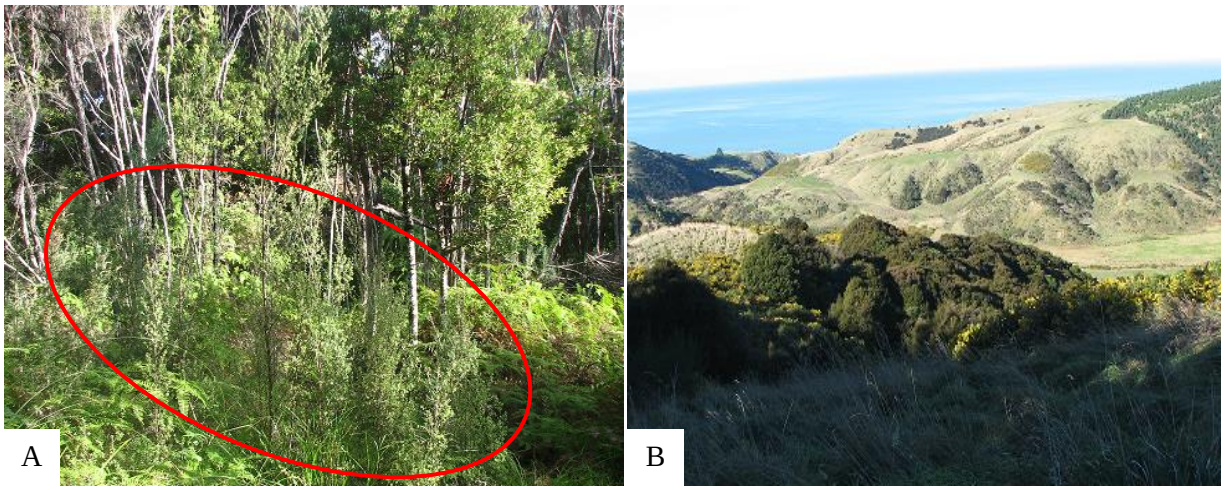

Figure 2.5. On the West Coast, (A) *K. ericoides* often coexisted with many other plant types, existing here as short understory plants. In Canterbury, (B) the lack of closed-canopy forest *means kanuka can form dense mature thickets with minimal undergrowth, seen here surrounded by yellow-flowered gorse.*

2.4.2 Mycorrhizal Colonization

Of the 86 sample seedlings, all (100%) had some mycorrhizal colonization present on their root systems. I observed that kanuka is much more likely to form a relationship with ECM than AMF, with 94.19% of the seedlings forming a prevailing association with ECM. And the association with ECM was stronger with the average colonization by ECM at 30.18% of the root system as opposed to AMF with an average colonization level of 12.64%. Only 5 seedlings had a larger AMF presence and overall, the roots had nearly double the amount of ECM occurrences (see Table 2.2). The micro-topography (See Table 2.3) within each site had no significant effect on the within-site levels of mycorrhizal colonization by AMF ($p=0.53$) or ECM ($p=0.48$), roots with no fungal presence ($p=0.93$), nor the total root count for each plant ($p=0.42$). This means that I can use all 86 samples to look at the effects of rainfall across the measured moisture gradient.

Table 2.2. n=86; The association between fungus type and seedling is shown, as well as the total number of roots counted. *K. ericoides* forms a stronger association with ECM. 12 of 86 seedlings had only ECM colonization and 2 of 86 had only AMF.

	Fungal association of each seedling	Colonization of seedling roots (%)	Total number of roots counted
Ectomycorrhizae	84 (97.7%)	30.18±2.98	2347
Arbuscular Mycorrhizae	74 (80.05%)	12.64±1.81	1205
No fungal presence	0	20.58±2.11	5110
Both types present	72 (83.7%)	42.82±2.33	348

Examining the average fungal colonization for each seedling against the expected rainfall, ECM colonization decreased non-significantly with higher rainfall, while AMF significantly increased (See Figure 2.6). AMF colonization was more variable than ECM in general, and was found to strongly positively correlated with rainfall, total manuka/kanuka coverage of the site, and slightly positively correlated with the slope of the site. AMF was significantly negatively correlated with altitude (See Table 2.4). The strong correlations with many different factors may be contributing to the overall inconsistency of AMF mutualism for *K. ericoides*. ECM was moderated negatively correlated with rainfall, and negligibly correlated with the other tested factors (See

Table 2.4). Both the total colonization levels and the number of "non-fungal" roots were only significantly correlated with increasing sample root counts (See Table 2.4), as expected with a higher root count. This means that an increase in the number of roots per sample increased the number of non-fungal roots, roots with AMF infection, and total fungal colonisation, but had no significant effect on the level ECM colonisation.

Table 2.3. Effect of micro-topography on: average root colonization by ECM and AMF, total number of roots per plant, and amount of non-infected roots. There was no significant difference in number of fungal infections or root growth due to the micro-topography within each sample site as calculated by the analysis of variance. 95% confidence levels used for ANOVA testing.

	Roots with ECM (Average)	Roots with AMF (Average)	Roots with no fungus (Average)	Total Root Count (Average)
Flat	29.37	17.67	59.40	106.43
Convex	23.36	11.75	57.21	86.17
Concave	29	12.36	61.64	96.17
ANOVA (p-value)	0.476 (not significant)	0.287 (not significant)	0.925 (not significant)	0.121 (not significant)

Table 2.4. This shows the **Pearson's r Correlation coefficient** between ECM, AMF, and total mycorrhizal colonisation with rainfall, altitude, and percent of site covered by *K. ericoides*. The significance of each was done using an ANOVA to calculate a p-value.

	Rainfall (mm) Correlation		Altitude (m) Correlation		Slope (Degrees) Correlation		Kan/Man Coverage (%) Correlation		Total Root Count Correlation	
	r	p	r	p	r	p	r	p	r	p
Ecto-mycorrhizae	-0.249	0.184	0.1	0.600	0.193	0.308	-0.138	0.467	0.058	0.76
Arbuscular mycorrhizae	**0.559**	0.001*	-0.446	0.014*	-0.147	0.438	**0.498**	0.005*	**0.653**	<0.001*
No Fungus Present	0.193	0.306	-.241	0.198	-0.024	0.9	0.354	0.055	**0.877**	<0.001*
Total	0.273	0.144	-0.303	0.104	0.036	0.849	0.316	0.09	**0.617**	<0.001*

Colonisation

(Moderate correlations are underlined, strong correlations are **bold**, *p<0.05)

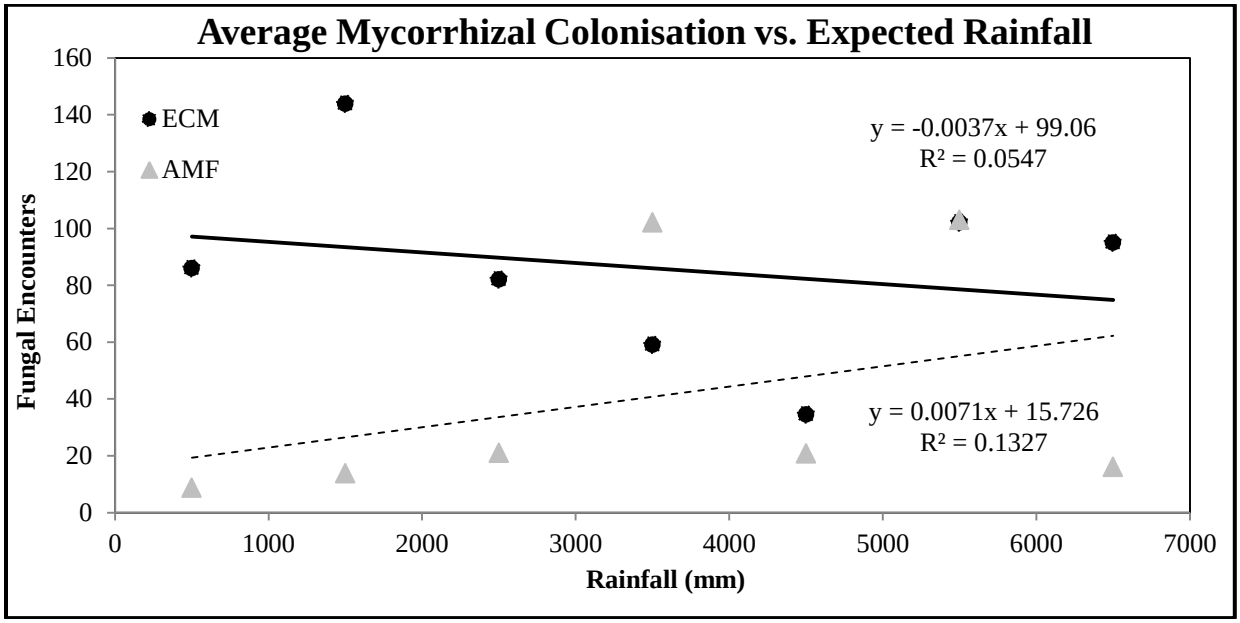

Figure 2.6: *Sampling K. ericoides across the large rainfall gradient of the South Island shows ECM colonization to have a weak negative correlation with increasing rainfall (r=0.25) and AMF colonization to have a strong positive correlation with increasing rainfall (r= 0.56, p=0.001).*

2.5 Field Survey Discussion

The main objective of the field survey was to verify where *Kunzea ericoides* associated with each arbuscular and ectomycorrhizae and to assess if rainfall levels are a determining abiotic factor of where each mutualism existed. The secondary aim was to measure if within-site colonisation variance could be attributed to the micro-topography of the plant location.

2.5.1 *K. ericoides* as a dual-mycorrhizal species

The field data confirm the wide range of *K. ericoides* and large-scale mycorrhizal relationships it forms, as all the sampled seedlings showed signs of root-fungal association. The ability of *K. ericoides* to form associations with both AMF and ECM was also supported. As all seedlings showed fungal association, both the ubiquity of these mutualistic connections and their fundamental role across the range of kanuka is established. Both types of mycorrhizae were present on the same individuals, showing that coexistence is possible on the same root system.

Only seedlings were sampled for this field survey, but several Australian eucalypt species were found to switch mycorrhizal partners from AMF to ECM as the plant matured (Chen et al. 2000, Adams et al. 2006). A more in-depth survey or growth experiment would have to be done to see if *K. ericoides* experienced a similar trend. The varying associations formed between mutualistic partners is based upon the relative benefit provided, supporting the idea that dual-mycorrhizal plants may have a larger than expected habitat range (Johnson et al. 1997, Bronstein 2001, Kummel and Salant 2006). Thus, the wide ecological success of *K. ericoides* could in part be due to its capacity to utilize each type of mycorrhizae where they are the most cost-efficient mutualistic partners.

2.5.2 Micro-topography and mycorrhizal colonisation

Within each site, the lack of mycorrhizal colonisation variation between the three micro-topography types supports the idea neither mycorrhizae type have trouble persisting in soil once becoming established. While micro-topography can have serious ramifications for seed germination (Harper et al. 1965, Simon et al. 2011), it does not seem to have much consequence when considering mycorrhizal presence below the soil surface, nor effect its ability to infect *K. ericoides*. Many soil nutrients and resources are extremely heterogeneous, which has caused plants to grow root systems for high levels of foraging (Wolfe et al. 2007, Zhang et al. 2010, Croft et al. 2012). This likely benefits mycorrhizae as far-reaching roots are more likely to encounter fungal patches in the soil and thus facilitate root colonisation. The lack of effect on root growth indicates that both these mycorrhizal mutualisms are more driven by the successful development of the plant roots rather than microsite conditions. As kanuka is a pioneer species, its ability to grow and establish mutualisms successfully under a wide variety of ecological circumstances, regardless of a particular micro-topographical profile, is logical.

2.5.3 Rainfall and mycorrhizal colonisation

The mutualism between *K. ericoides* and ECM is not significantly correlated with rainfall levels on the South Island of New Zealand, but AMF mutualism was shown to be significantly positively related. This confirms our hypothesis that arbuscular mycorrhizae colonisation increases in areas of high rainfall. Also, this supports previous findings of lowered rainfall and moisture levels causing a reduction of the fitness and activity of AMF (Liu et al. 2012).

specifically in New Zealand, podocarp forests with similar composition and structure both hosted similar levels of AMF colonisation regardless of rainfall, soil type and available light variability (Hurst et al. 2002). Yet, this project was restricted by forest type (podocarp), which may not indicate the true variability of AMF presence across a rainfall gradient as my thesis tried to demonstrate.

However, my data show that even at the highest recorded rainfall amounts, AMF colonisation only matches the level of ECM presence. This supports the idea that ECM is still the preferred mutualistic partner for *K. ericoides*, but it simply does not exclude AMF under high soil moisture conditions. It may be that ectomycorrhizae simply have a higher colonisation potential even at low soil concentrations. With ECM forming the majority of its mutualisms with woody species (Smith and Read 2010), they may respond more quickly than AMF to the woody roots of *K. ericoides*.

On the West Coast *K. ericoides* was often found concurrently with manuka. As AMF significantly increased with increasing manuka coverage, the higher AMF levels could be due to manuka being a more suitable host plant, leading to higher arbuscular mycorrhiza presence in the soil, thereby higher colonisation for the nearby kanuka. The mutualism between AMF and manuka was not the focus of this project, but manuka is known to grow more successfully than kanuka in areas of high moisture (Wilson and Galloway 1993). Also, the relationship between *K. ericoides* and AMF could be an evolutionary remnant from before the existence of ectomycorrhizae (Smith and Read 2010), potentially signifying that the relatively new ECM mutualism is specific to kanuka rather than being a generalist relationship. This specificity could indicate a more robust relationship between *K. ericoides* and its ectomycorrhizal colonisers than AMF.

Although this project did not focus on the specific fungal species forming the mutualism with kanuka, this tree showed a greater affiliation with ectomycorrhizal fungi, and ECM have been found to dominate fungal mutualisms at lower soil moistures (Lodge 1989, Khan 1993) As this ECM-kanuka mutualism was not significantly affected by any of the measurements taken, many other issues that may be contributing to the relative dominance of ECM colonisation.

It has been suggested that ectomycorrhizae may cause exclusion of non-ECM plant species by changing soil nutrient cycling, lowering plant diversity, and therefore establishing habitat dominance of certain species (Dickie et al. 2014). Thus, it is arguable that as a pioneer species *K. ericoides* may perpetuate ECM colonisation when it first establishes, then excludes other species from mature stands, contributing the competitive advantages of ECM. This seems feasible for Canterbury sites where *K. ericoides* scrub was found to be a major woody cover in areas not dominated by grassland. This exclusion factor may be contributing to the increased presence of kanuka species in low rainfall, along with its high drought tolerance and higher root colonisation of ECM under low moisture conditions (See Chapter 3) (Innes and Kelly 1992). The presence of AMF even at low rainfall may suggest that hosting both mycorrhizal types may augment the nutrient benefits provided by each type. From the plant's perspective, an ectomycorrhizal mutualism may be more physically advantageous in low moisture soils. The hardened soil structure in these dry soils affect the ability of plant roots, due to their size, to physically expand through the substrate (Lodge 1989, Smith and Read 2010); this leaves seedlings dependent on the smaller ECM external hyphae to access both moisture and soil nutrients. This external matrix is not present to the same extent for arbuscular mycorrhizae (Smith and Read 2010). It is also possible that the timing of carbon substrate production (via root transfer or leaf litter) matches the requirements of ECM more closely than AMF, leading to a more efficient relationship with ectomycorrhizae (Dickie et al. 2010). Ectomycorrhizae may be excluding the arbuscular mycorrhizal species physically with the hyphal sheath and external mycelia acting as a barrier around the roots. As mycorrhizal infection can only develop in new roots (Chen et al. 2000, Smith and Read 2010), it is possible that lowered root growth and turnover due to water stress reduces secondary AMF establishment by decreasing the amount of available habitat. It is also possible that there is higher competition between the two fungal types at low water availability. This could be direct hyphal antagonism between the two, heightened competition for carbon resources, or ectomycorrhizae may induce changes in root exudates to increase resistance to subsequent AMF infection. It is unknown the extent of any or all of these factors contributing to the relationship between ECM and AMF competition.

2.5.4 ECM and the West Coast Beech Gap

For areas on the West Coast, samples were also taken across the border of one New

Zealand's "Beech Gaps". This is an area on the west cost of New Zealand's South island which is notoriously absent of beech trees, but comprises of habitat that is normally suitable for *Nothofagus* species. It is thought that the beeches were originally excluded by the latest glaciations period occurring 34,000-18,000 years ago (Trewick and Wallis 2001, Leschen et al. 2008). Currently, there no complete answer to why this area still lacks beech along 150 km of the Westland coast, but low dispersal ability is the most likely a contributing factor (Orlovich and Cairney 2004, Dickie et al. 2012). One argument is that ECM, which dramatically increases the establishment ability of beech species, especially into manuka, is not present or at a negligible level in this area, preventing the spread of these trees into the beech gap (Dickie et al. 2012, Pena et al. 2013). This reduced amount of ECM is reflected slightly in the field data, but the measured levels did not dip below recorded AMF quantities. Direct measurements of ECM presence in the soil were not taken, but the colonisation levels of plant roots are likely to reflect soil concentration. The decline in ectomycorrhizal occurrence may also be due to site choice, as soil resources are known to be highly heterogeneous (Huante et al. 1998, Merryweather and Fitter 1998b, Kozlowski and Pallardy 2002, Lamb et al. 2012). So, while the increase of AMF is correlated with rainfall, the lowered ECM presence may not be due to competitive ability, rainfall amounts, slope of the site or altitude, but instead is due to historical precedence.

The data collected by this project do not provide all the answers, but offer a glimpse into the real-world relationship between *K. ericoides* and its mutualistic partners. This field survey confirmed the complexity of mycorrhizal mutualisms, with both fungal types colonising *K. ericoides* in both high and low rainfall areas. And it corroborated the dominance of the ectomycorrhizal mutualism in low soil moisture and arbuscular mycorrhizal colonization increasing with high rainfall. More research needs to be done in relation to the soil types and their effect on mycorrhizal functioning in New Zealand. This assessment did not focus on the soil characteristics, instead hoping to find a more straightforward measurement which could be used to predict fungal mutualist presence. By hosting both mycorrhizal types, *K. ericoides* has the ability to provide the necessary rhizospheric facilitation for either ECM or non-ECM species, demonstrating its importance as an ecological pioneer. However, with the recent elucidation of the genus *Kunzea* (de Lange 2014), it is currently unknown if the newly named species preferentially interact with certain mycorrhizal species, affecting its ability to facilitate later successional plants. Overall, this field research confirmed specifically the wide range of and the

mutualistic relationships for *K. ericoides* and both arbuscular and ectomycorrhizae. These results allow the following glasshouse experiment to quantify the relationships and further clarify their physiological role.

Chapter 3 The Glasshouse Experiment

This chapter describes the growing of *K. ericoides* from seed under varying watering levels with or without inoculation with ECM. The aims are to (1) assess how root colonisation by ectomycorrhizae varies with soil moisture, (2) measure the effect of ECM colonisation on the growth of *K. ericoides*, and (3) determine if differing colonisation levels change the water stress experienced by *K. ericoides* seedlings. By measuring how ECM effects the establishment phase of *K. ericoides*, we can begin to understand the ecological ramifications of ectomycorrhizal mutualisms across abiotic gradients and unravel the important factors which determine mutualism functioning. We propose that as soil moisture decreases, ectomycorrhizal colonisation will increase and at this higher colonisation level will increase access to nutrients and soil moisture, causing ECM inoculated *K. ericoides* seedlings to grow larger. Also under low water availability, ectomycorrhizal colonisation will cause a delay in experienced water stress for the seedlings due to an increased ability to obtain water.

3.1 Germination of *K. ericoides* Seedlings

Working with kanuka in the greenhouse is often successful (Burrows 1996). Problems with growing from seed arise from the drop off in seed viability in relation to freshness, or improper placement of seeds in the soil. Kanuka readily germinates on bare soil in the field and also in sparsely populated grass covering (Allen et al. 1992). In the greenhouse, kanuka seeds have up to 100% rate of germination success as fresh seed when washed in tap water and sprouted on a moist filter paper, and a 92% success rate after 5 months of dry storage (Burrows 1996, Rowarth et al. 2007). Appropriate for its role as a colonizing species, *K. ericoides* germination is completely stopped by seed burial and severely inhibited in shaded environments (Burrows 1996). One important reason for working with kanuka as opposed to manuka in the Canterbury region is the avoidance of manuka blight, which is caused by an invasive scale insect and can devastate entire experiments (Stephens et al. 2005).

3.2 Assessment of mycorrhizal interactions

When studying any type of biotic interaction, it is important to consider the variability in incidence and strength. Currently, there is a research demand to move away from "if" mycorrhizae affect plant community to "how much" and to carry out measurements of these interactions (Orlovich and Cairney 2004, Klironomos et al. 2011). Studying soil mycorrhizae has its challenges and limitations simply due to the system and size of the organisms, but also due to incredible amount of spatial variability. Mycorrhizae are known to be heavily affected by abiotic factors (e.g. moisture, temperature), making selection of sampling sites for field study fairly risky (Klironomos et al. 2011). Looking along an environmental gradient and considering large sampling numbers can mitigate some of this risk, but translates into many hours of delicate lab work. Glasshouse experiments can be manipulated more fully, but there can be difficulties in soil inoculation; especially if the manipulated soil is unsuitable for mycorrhizal growth (Klironomos et al. 2011, Davis et al. 2013). However, in glasshouse, environmental factors can be singled out successfully to make strong conclusions.

3.3 Experimental design

The glasshouse experiment was designed to quantify how the association with different mycorrhizae would affect the *K. ericoides* seedling growth under drought conditions. The inclusion of this controlled trial allows for a more coherent quantification of the mycorrhizal mutualism on the establishment and growth rate of *K. ericoides* under a similar range of moisture conditions as those that exist in nature.

The experimental groups of seedlings were grown in soil inoculated with both ECM and AMF. The control groups were grown in soil containing only arbuscular mycorrhizae. Two trials were simultaneously carried out with two types of soil, one type taken from underneath a stand of manuka trees, and the second soil type was taken from a site with only kanuka present (see Table 3.1). Both of these soils are from the University of Canterbury CASS Alpine Research Station. From results in the field experiment, these two soils were known to contain ECM and AMF and were used as the inoculation for the experimental groups. The control "inoculate" was from the same sites as the ECM inoculates, but was sterilized by autoclave before being combined.

By replicating the experiment in the two soil types, I hope to more accurately imitate the natural range of conditions in which *K. ericoides* would grow. As manuka and kanuka often form mixed stands, assessing the effects of soil parentage on ECM will give a more complete picture of how this mycorrhizal mutualism adjusts due surrounding species. While there will be no direct competition between manuka and kanuka, residual soil characteristics or different mycorrhizal species will present differently in the growth effects for *K. ericoides*.

The growth medium soil was taken from a kanuka and manuka free site near Mt. Barker (Raikaia, Canterbury). This medium was not sterilized and was assumed to contain AMF. The top 5 cm of soil was removed to reduce the chance of any ECM infection of the *K. ericoides* seedlings. Before combining, all soils were sieved through a 1 cm grid to break down large soil clumps and remove rocks and other debris. This size maintains some soil characteristics and allows root fragments to act as mycorrhizal carriers to the seedlings. The growth medium was combined with 15%, by weight, of the ECM inoculate or control "inoculate".

Table 3.1. Sites where soil was taken for ECM inoculate. Both are alpine soils.

Species	Site Name	Location	Latitude	Longitude	Altitude
Manuka	CASS - Station	Waimakariri catchment	-43.037582	171.773043	567
Kanuka	CASS - Sugarloaf	Waimakariri catchment	-43.030068	171.788401	1018

The watering regime was determined by calculating the field capacity (FC) of each combination soil type. Soil field capacity is the amount of soil moisture held in soil after excess water has been drained away, causing the rate of downward water movement to decrease (Israelson and West 1922). The field capacity calculation was done by saturating a sample of the soil mixtures and then allowing them to dry while taking careful measurements of the rate of water loss (Israelson and West 1922). The groups were watered to either saturation, to field capacity (FC) or to half of field capacity (FC/2). This was combined with different watering schedules of a 4 day, 8 day, 12 day or 16 day to emulate different rainfall amounts (See Table 3.2). This created eight different watering groups, replicated in the control set. To compensate for temperature variation in the glasshouse environment, the groups were grown in randomized blocks, with plants from each soil group present in the eight blocks. There were eight seedlings grown for each watering and ECM inoculation scenario, leading to 64 seedlings in each the experimental

group and control group. These two trials were carried out simultaneously in the alpine glasshouse at the University of Canterbury on adjacent benches.

Kanuka (*Kunzea ericoides*) seeds were purchased and germinated on paper placed over sterile peat to maintain moisture. The temperature was regulated to a 30 degrees Celsius using a germination cabinet with light cycles mimicking the summer sun schedule for the South Island. The germination rate was incredibly high with this method, similar to the 98% success rate proposed by Burrows (1996). The *K. ericoides* seedlings were transferred to the pots of mixed soil (3 per pot) when large enough to safely move (5-8 mm). One seedling was removed at the 3-month and 6-month mark to confirm ECM and AMF inoculation for the various groups. The pots were re-randomized twice during the growing period (See Fig 3.1).

Table 3.2. Description of glasshouse experiment. Each watering schedule has one experimental group (mycorrhizal inoculation with ECM and AMF) and one control group (mycorrhizal inoculation with only AMF). This setup is repeated for two soil types, manuka-soil and kanuka-soil.

Group number	Watering Schedule	Mycorrhizal Treatment	Soil Types	
1	Every 4 days to soil saturation	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka
2	Every 4 day to soil field capacity	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka
3	Every 8 days to soil saturation	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka
4	Every 8 days to soil field capacity	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka
5	Every 8 days to half soil field capacity	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka
6	Every 12 days to soil field capacity	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka
7	Every 12 days to half soil field capacity	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka
8	Every 16 days to half soil field capacity	ECM+AMF	manuka	kanuka
		AMF (Control)	manuka	kanuka

Figure 3.1. Picture of *K. ericoides* seedlings in during growth experiment. Picture taken during first re-randomization of treatment blocks.

3.3.1 Analysis

After nine months of growing, each group was harvested and the total ectomycorrhizal colonization was counted using a modified gridline intercept method and the same methods of ECM identification as used for the field experiment (see Chapter 2) (Dickie et al. 2012). The pots were soaked in water to gently loosen the soil from the roots and then examined for ECM (see Fig. 3.2). 100 root tips were counted for each seedling and classified as either with or without ECM colonisation. Once ECM presences was determined, the height of the plants was measured the above-ground biomass (leaves and stem) were separated from the below ground biomass (roots) and were dried over three days in a 70 degree Celsius drying oven. Both plant sections were weighed to determine how the ECM colonization affected the growth patterns of the seedlings. Several sample plants were also examined for presence of arbuscular mycorrhizae, which was, as predicted, found across all groups. Quantifying the AMF colonisation was not part of this experiment and was assumed as a baseline condition.

Statistical analysis of the results was done using R. All results were tested to for normality to ensure the baseline analysis assumptions were met. Statistical differences in height, root/stem biomass, and water usage were calculated using an analysis of variance (ANOVA). Pair-wise mean comparisons were done using the Tukey-Kramer method .

Figure 3.2. Example of *K. ericoides* seedling after harvest and root cleaning. This seedling was grown in manuka-soil and inoculated with ECM. Picture was taken just before root tips were examined under magnification.

3.4 Results

All but one pot of seedlings seemed to grow satisfactorily (255 seedlings at harvest). There were some herbivory issues with caterpillar infestation. These were removed as soon as noticed. The seedlings grown under the driest conditions showed signs of being near the limit of survival as there was a minor level of leaf death during the hottest growing periods. Soaking the plants in water for at least 30 minutes was essential to removing hardened soil from the root systems, especially for the seedlings grown under very low soil moisture conditions.

3.4.1 ECM Colonisation

Identification of ectomycorrhizae was clear across inoculated groups, with the external mycelia and hyphal sheaths readily apparent under low magnification. The non-colonised roots were smooth and nearly white in colour, while the colonised roots were wrapped in darker fungal hyphae and looked fuzzy. All of the seedlings grown in ECM inoculated soil showed signs of ECM colonisation. Seedlings grown in the control group (no ECM inoculation) did not show any sign of ECM presence on root tips as determined by examining 1 seedling from each watering group. Across all watering groups, the average number mycorrhizal root tips out of 100 were 34.03 and 38.1 for seedlings grown in manuka- and kanuka-soil respectively. For both soil types, the ectomycorrhizal colonisation of the seedlings significantly increased as soil moisture decreased (see Fig 4.3, $p<0.001$ for both). For both soil types, a drastic increase in ECM

amounts occurred between watering group 4 and 5. There was no significant difference in ECM colonisation between the two soil types for any of the watering groups. Both manuka- and kanuka-soil seedlings followed similar patterns of ECM increase.

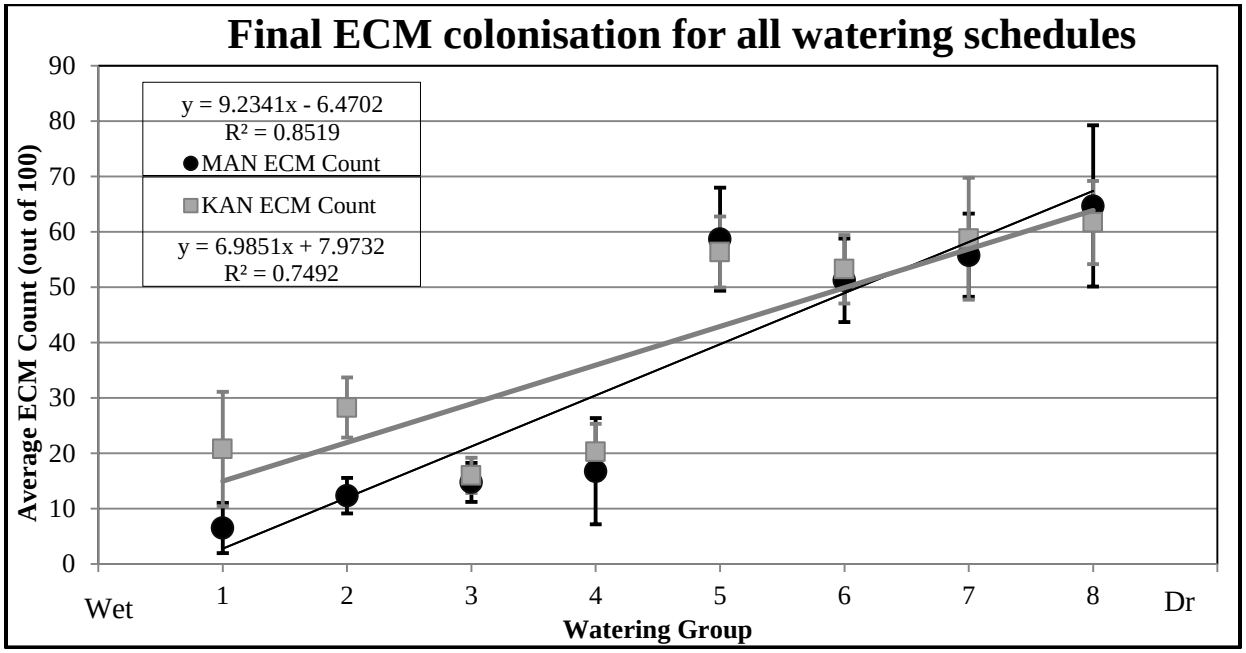

Figure 3.3. Average number of root tips encountered with ECM colonisation for the different water groups. ECM colonisation increases dramatically when half of the soil field capacity is the maximum level of soil moisture for both soil types. Error bars show the variance for each of the groups. 100 root tips were counted for each seedling (800 per watering group)

3.4.2 Seedling height and growth rates

All seedlings grew quickly when first potted, then slowed during the colder winter months. Spring brought an increase growth rates across all treatments. See Appendix 1 for the full set of height versus time charts for all treatment groups. Height measurements were taken from where the seedlings emerged from the soil to the tip of the top leaf. The amount of growth was measured on each scheduled watering day for each group.

ECM inoculation had mostly a negligible effect on the final height of *K. ericoides* seedlings grown in the kanuka-soil type. The average final heights across all watering groups, for seedlings grown in kanuka-soil were 14.34 cm and 18.03 cm for the ECM treatment and control groups respectively. The tallest seedlings grew in watering group 2 (watering every 4 days to soil saturation) for both the ECM and control seedlings. This control group contained the tallest seedlings for the entire experiment, but only grew significantly larger than the ECM treatment in

watering group 2 (See Table 3.3). When growing in drought conditions, there was no significant difference between the height of the ECM and control treatments. The average monthly growth rates for the kanuka-soil groups were 1.53 cm and 1.95 cm for the ECM and control treatments respectively. Only the driest growing condition (watering group 8) showed the ECM inoculated seedlings growing significantly faster than the control group (See Table 3.4).

For *K. ericoides* seedlings grown in manuka-soil, ECM inoculation had a mostly negligible effect on the final height. Across all watering groups, the average final heights were 19.65 cm and15.96 cm for the ECM treatment and control groups respectively. The tallest seedlings were in watering group 3 for the ECM treatment and in watering group 4 for the control seedlings. Watering group 1 was the only scenario that had a significant difference in average final height when compared to the control group, with the ECM group growing taller (See Table 3.3). Only in the wettest soil, did ECM inoculation significantly increase the height of the seedlings, with no effect in drought conditions. The average monthly growth rates for the manuka-soil group were 2.01 cm and 1.40 cm for the ECM and control groups respectively. There was no significant difference in growth rates caused by ECM inoculation for manuka-soil seedlings (see Table 3.4)

Comparing across soil type, the manuka-ECM treatment produced taller seedlings for all watering groups except the driest. However, this height difference was only significant for watering group 1 (See Table 3.3). Again, only the wettest growing conditions showed significantly increased height of ECM-inoculated seedlings. Comparing control groups, no significant difference was found for any of the watering regimes, supporting the idea that soil type alone does not affect the height of *K. ericoides* seedlings.

Table 3.3. Average heights at final harvest for all treatment groups. For the wettest scenario, the seedlings grown in manuka-type soil with ECM inoculate grew significantly larger than both the control group ($p<0.05$) and the kanuka-soil type with ECM ($p<0.05$). ECM inoculation showed no significant effect on the final height of seedlings when grown under drought conditions.

Watering group: Schedule	MAN-ECM Average final height (cm)	MAN-Control Average final height (cm)	KAN-ECM Average final height (cm)	KAN-Control Average final height (cm)
1: 4 day to saturation	20.75*, **	12.6	12.08	15.03
2: 4 day to field capacity	20.17	17.2	19.98	25.8^
3: 8 day to saturation	22.65	15.53	14.88	19.85
4: 8 day to field capacity	18.6	18.8	15.93	21.55^
5: 8 day to half field capacity	19.17	15.87	12.8	18.4
6: 12 day to field capacity	22.2	16.93	14.78	13.65
7: 12 day to half field capacity	18.85	16.68	13.88	16.3
8: 16 day to half field capacity	13.2	13.43	8.63	12.3

(*$p<0.05$ for ECM group larger than its control group, **$p<0.05$ for MAN-ECM larger than KAN-ECM, ^$p<0.05$ for control group larger than ECM group)

Table 3.4. Average growth per month of *K. ericoides* seedlings for all treatment groups. The kanuka-soil ECM group grew significantly faster than the control group under the driest conditions.

Watering group: Schedule	MAN-ECM Avg monthly growth rate (cm)	MAN-Control Avg monthly growth rate (cm)	KAN-ECM Avg monthly growth rate (cm)	KAN-Control Avg monthly growth rate (cm)
1: 4 day to saturation	2.24	1.49	1.50	1.85
2: 4 day to field capacity	1.95	1.40	2.29	3.04
3: 8 day to saturation	2.38	1.65	1.59	2.45
4: 8 day to field capacity	1.79	1.44	1.55	2.69
5: 8 day to half field capacity	2.02	1.76	1.34	2.15
6: 12 day to field capacity	2.00	1.17	1.22	1.35
7: 12 day to half field capacity	1.76	1.30	1.26	1.48
8: 16 day to half field capacity	1.90	1.02	1.45*	0.57

(*$p<0.05$ for ECM group larger than its control group)

3.4.3 Seedling Biomass

The above and below ground biomasses were attained after drying of *K. ericoides* seedlings to remove the water weight. The effect of ectomycorrhizal colonisation on root biomass was very pronounced for the seedlings grown in manuka-soil. This treatment group was produced the largest root systems for the whole experiment across all watering groups. The manuka-soil ECM

root biomasses were significantly larger than the control seedlings for all watering groups except group 8; and, they were significantly larger than the kanuka-soil ECM for all watering regimes except group 5 and 8 (See Table 3.5). For the kanuka-soil seedlings, ECM inoculation did not significantly augment root system size for the majority of the watering groups. Only the lowest soil moisture groups showed increased root system size when compared to the control group. This indicates that for root system size, there was some residual effect was due to soil type rather than only responding to colonisation by ectomycorrhizae.

Table 3.5. Average root biomass at final harvest for all treatment groups. The large size of the root systems grown by the manuka-ECM group suggests that the soil type caused the significant differences to occur, possibly through soil structure or nutrient level disparities.

Watering group: Schedule	MAN-ECM Avg root biomass (mg)	MAN-Control Avg root biomass (mg)	KAN-ECM Avg root biomass (mg)	KAN-Control Avg root biomass (mg)
1: 4 day to saturation	393*, **	87.5	158	100
2: 4 day to field capacity	453*, **	175	308	228
3: 8 day to saturation	383*, **	138	95.0	165
4: 8 day to field capacity	380*,**	203	190	190
5: 8 day to half field capacity	303*	150	200	237
6: 12 day to field capacity	303*, **	120	190	180
7: 12 day to half field capacity	285*,**	160	188*	110
8: 16 day to half field capacity	177	147	167*	86.7

(*$p<0.05$ for ECM group larger than its control group, **$p<0.05$ for MAN-ECM larger than KAN-ECM)

Measuring the root to shoot ratio shows how much energy the plant devoted to each part as a result of either water stress or ECM inoculation. ECM colonisation caused the plants to devote more energy resources to root system growth. For the seedlings grown in kanuka-soil, across all watering groups there was a significant increase in the root to shoot ratio when inoculated with ECM ($p<0.05$). In the kanuka-soil seedlings, the total root biomass averaged 37.28% and 23.45% of the total plant for the ECM and control treatments respectively (See Table 3.6). The relatively bigger root systems partially reflect the larger height of the kanuka-control seedlings.

Significance was not achieved ($0.05<p<0.1$) for the root to shoot ratio of the manuka-soil ECM treatment and the control groups when compared as a whole (see Fig. 3.4). This shows that ECM inoculation alone did not cause the root to shoot ratio to increase. In the manuka-soil seedlings, the root biomass averaged 32.79% and 27.36% of the total plant for ECM and control treatments respectively (See Table 3.6). However, when comparing the different watering groups, there was a significant difference for the ECM inoculated seedlings when high water

stress was also occurring (See Table 3.6). There was no significant difference in root system size between the manuka-soil and kanuka-soil ECM groups. This implies that the differences root system sizes were not due to soil type, but due to ECM colonisation. This root response was amplified by water stress.

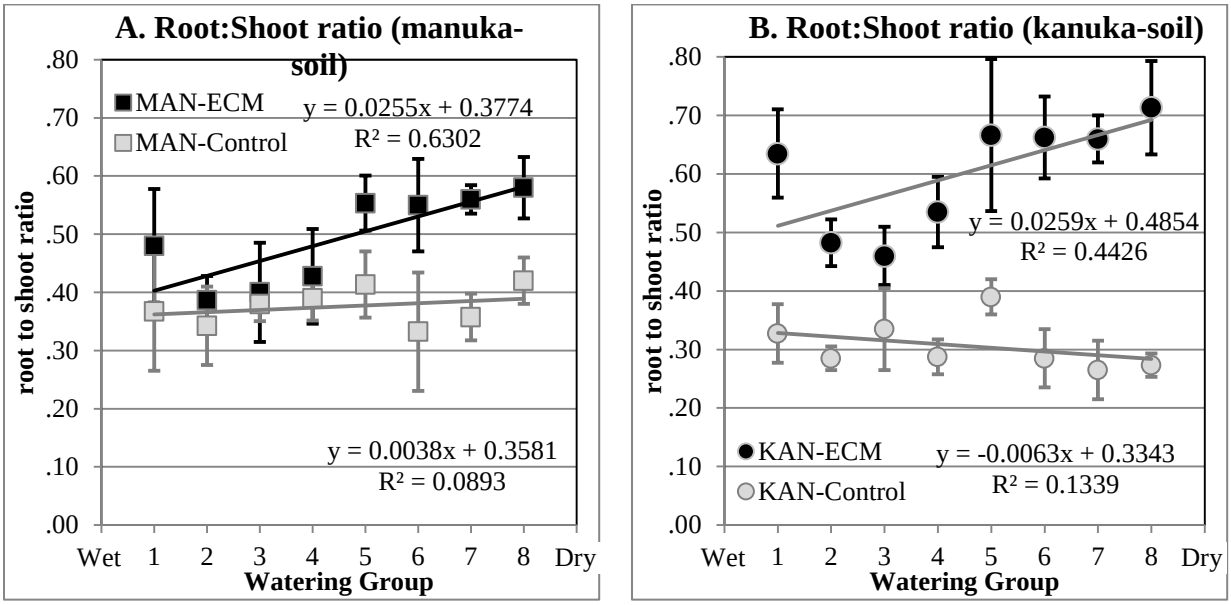

Figure 3.4A-B. Side-by-side comparison of root to shoot ratio for manuka (A) - and kanuka-soil (B) groups. ECM inoculation increased the amount of energy devoted to root system growth for both soil types, but was only significantly increased the root system size for the kanuka-soil group ($p<0.05$).

Table 3.6. Weight of root systems as a percentage of the whole plant. For the kanuka-soil group, ECM inoculation caused a dramatic increase in the size of the root system across all levels of soil moisture.

Watering group: Schedule	MAN-ECM Root %	MAN-Control Root %	KAN-ECM Root %	KAN-Control Root %
1: 4 day to saturation	32.57	27.13	39.38^	25.00
2: 4 day to field capacity	27.93	25.27	32.28^	22.14
3: 8 day to saturation	28.60	27.50	32.20*	25.29
4: 8 day to field capacity	30.10	27.93	34.86^	22.09
5: 8 day to half field capacity	35.83*	29.41	38.71*	28.06
6: 12 day to field capacity	35.17*	25.53	39.58^	22.57
7: 12 day to half field capacity	35.85^	26.34	39.89^	20.85
8: 16 day to half field capacity	36.30*	29.73	42.32^	21.67

(*$p<0.05$ for ECM group larger than its control group, ^$p<0.001$ECM group larger than its control)

3.4.4 Seedling Water Usage

Similar to height, water usage was high after potting, lower during the winter months, and again increased during the warmer spring. See Appendix 2 for the full set of water usage versus time charts for all treatment groups.

The ECM inoculation had a significant effect on water usage for seedlings grown in dry conditions. For both soil types, seedlings inoculated with ECM used significantly less water per day on average than the control groups (See Table 3.7, $p<0.05$ for both soil types). For seedlings grown in kanuka-soil, daily water usage was significantly higher for the ECM group grown the wettest soil (group 1, $p<0.001$). Daily intake of water was significantly higher for watering groups 2, 3, and 8 for the kanuka-soil control group (see Table 3.7). For manuka-soil types, water usage per day was only significantly higher for the control group under the driest soil condition ($p<0.05$).

When comparing across soil types, the manuka-soil tended to use more water when high amounts of water were available. This supports the idea of some legacy left by the adult manuka affecting the water usage of the *K. ericoides* seedlings. Overall, both soil types showed increased water usage when inoculated with ECM for high water scenarios.

Table 3.7. Average daily water usage of *K. ericoides* seedlings for all treatment groups. Under high water stress ECM inoculation caused the seedlings to use significantly less water. The high water usage by the ECM inoculates at high water availability suggests ECM colonisation can adapt to either high or low moisture availability.

Group: Watering Schedule	MAN-ECM Average daily water usage (mL)	MAN-Control Average daily water usage (mL)	KAN-ECM Average daily water usage (mL)	KAN-Control Average daily water usage (mL)
1: 4 day to saturation	4.40	3.82	5.87*, ^	4.07
2: 4 day to field capacity	5.38^^	4.31	3.29	4.27*
3: 8 day to saturation	4.62^	4.23	3.32	4.55*
4: 8 day to field capacity	4.17^	4.18	3.12	3.52
5: 8 day to half field capacity	3.54	3.24	2.99	3.63
6: 12 day to field capacity	3.61	3.44	2.86	3.22
7: 12 day to half field capacity	3.20	3.05	2.65	3.01
8: 16 day to half field capacity	2.80	3.43*	2.89	3.48*

(*$p<0.05$ higher water use compared between inoculation treatment, ^$p<0.05$ higher water use compared between soil type, ^^$p<0.001$ higher water use compared between soil type)

3.5 Glass House Discussion

The main objective of the glass house experiment was to determine how ectomycorrhizal colonisation affected the establishment and growth of *Kunzea ericoides* under varying water stress. The secondary aim was to see how soils taken from manuka (*Leptospermum scoparium*) and kanuka (*K. ericoides*) affected how the seedlings responded to ectomycorrhizal inoculation.

3.5.1 Ectomycorrhizal inoculation and above-ground biomass

From the results, we see that ectomycorrhizal inoculation had the greatest effect on *K. ericoides* seedling growth under extremely dry growing conditions. As expected, the seedlings experienced drought stress and reduced growth under the low watering schedules. While *K. ericoides* is highly drought tolerant (Innes and Kelly 1992), mycorrhizal fungi have been shown to grow at water potentials below the minimum for growth of the host plant (Desprez-Loustau et al. 2006). This is supported by the increasingly successful colonisation by ECM of the seedlings and the high density of mycorrhizal root tips encountered under drought conditions. However, even when ectomycorrhizal colonisation levels reached 60% of the entire root system, which occurred only under extreme drought conditions, there was no effect on total above ground biomass production.

Since the effect by ECM infection was mostly detrimental to the final height of the seedlings, it supports the proposal that along abiotic gradients, mutualist partners can act as parasites under certain environmental circumstances (Johnson et al. 1997). Normally, plant growth promotion by mycorrhizae is based upon improved nutrient access, increased moisture acquisition, and possible hormonal stimulation (Berg 2009). It is possible that the ECM species that colonise *K. ericoides* may need longer than the measured growth period before the mutualism is actually mutually beneficial. Since mutualistic relationships function by trading assistance, mycorrhizal colonisation can represent a significant drain on carbon supply to the plant (Brundrett 1991, Bronstein 2001, Smith and Read 2010). The carbon produced through photosynthesis must be shared between the fungi and plant, rather than all of this resource being devoted to plant growth. This leads to a reduction of the measurable positive growth effects, causing the benefits of higher nutrient and moisture uptake by the ectomycorrhizae to be lacking or negligible. And the high concentration of ECM at low moisture levels may amplify this parasitic effect. As the control group (only AMF present in the soil) grew larger for the seedlings grown in kanuka-soil, the

drain of carbon resources by ECM seems to have retarded the overall stem and leaf production of the seedlings. Other research has shown that ECM colonisation does not necessarily guarantee enhanced growth, and the positive effects are incredibly variable depending on the colonizing species, the host plant biotype, and the possible presence of other mycorrhiza (Smith and Read 2010). This variation of mutualism benefit is mirrored by the height data from this experiment, but, there dominant presence of ECM in low moisture conditions hints that a beneficial relationship may develop once the plant is larger or when higher levels of colonisation are reached.

3.5.2 Root system growth

The root systems of the seedlings were a significantly larger proportion of the plant when grown in ECM inoculated soil. Root to shoot ratio rather than absolute biomass is important as ECM can contribute a non-negligible amount to the weight of the roots and it is nearly impossible to accurately weigh the ECM separately from the root system (Smith and Read 2010). This increase of root percentage is somewhat contrary to previous studies in which the mycorrhizal hyphae somewhat substitute the root system function, meaning that the plant can allocate resources to above ground biomass production while depending on the mycorrhizal system to supply a larger proportion of the water and nutrient uptake (Chen et al. 2000, Croft et al. 2012). For *K. ericoides*, ECM colonisation caused the root system growth to surpass one third of the total plant weight.

Larger root systems are important in seedling survival, allowing the plant to take advantage of the spatial and temporal heterogeneity of soil nutrients and moisture (Huante et al. 1998, Zhang et al. 2010, Croft et al. 2012, Lamb et al. 2012). The difference in root system growth in response to ECM inoculation demonstrates high morphological plasticity, which suggests *K. ericoides* is able to successfully grow in a wide range of moisture and nutrient levels. Increasing allocation of resources to root growth is often also a response of woody species in drought conditions to support the nutrient and water requirements of the above ground structures (Kozlowski and Pallardy 2002). Early successional species have been shown to derive greater benefit from rapid soil exploitation than late successional species (Huante et al. 1998) and as *K. ericoides* is a pioneer species, it is likely to establish in problematic habitats where harsh soil conditions have not yet been ameliorated. The ability to rapidly expand their root system as

seedlings into high nutrient soil patches promotes establishment priority when newly disturbed habitat becomes available. This fast development was apparent in the significantly higher growth rate of *K. ericoides* under severe drought conditions (See Table 3.4). The partnership with ECM enhances the foraging capacity of *K. ericoides* by increasing the responsiveness of the root system, and therefore promoting greater success of new individuals. Another benefit of larger root systems is that in harsh conditions, an increase in root system size can promote favourable soil conditions to develop by improving subsoil structure, thus allowing future generations to become more easily established (Cresswell and Kirkegaard 1995). Also, ECM can support larger root system growth in some beech tree species, allowing them to endure drought conditions more easily (Pena et al. 2013), thus this mutualism between *K. ericoides* and ECM extends beyond the kanuka tree to benefit the entire ecosystem. The relationship that exists between *K. ericoides* and ectomycorrhizae strengthens this species ecological profile as an important pioneer species in New Zealand ecology.

3.5.2 Effects of soil type

The two soil groups provide a compelling comparison of *K. ericoides* across its range of habitat possibilities. The legacy of each parent plant (manuka or kanuka) caused different responses to ECM inoculation.

The lack of vigour of the ECM inoculated seedlings grown in the kanuka-soil presents an example of the negative effects of mutualisms. Even though the ECM inoculate from this specific soil would be most beneficial to *K. ericoides* seedlings, the carbon drain of the dual-mycorrhizal mutualism dampened the above-ground biomass production. This energy loss to ECM was evident considering the kanuka-soil control seedlings (no ECM inoculate) were the tallest treatment group. As the growth time was only 9 months, it is possible that the seedlings hat not yet reached the state where greater stem and shoot size was central to survival. However, with this soil type, the ECM colonization significantly increased root growth rate and significantly decreased water usage for the driest conditions for the seedlings.

The increase of the root to shoot ratio was significant across all levels of water availability for the inoculated seedlings grown in kanuka-soil, but only for the driest condition in the manuka-soil group. The focus of energy on the root systems for the kanuka-soil type indicates that when growing near a parent of the same species, there could be heightened below-ground competition

between the two. This is suggestive of a negative Allee-type effect, where the density of a population reduces the mean fitness of individuals (Berec et al. 2007, Courchamp et al. 2008). Growing near an adult conspecific, the two individuals would have the exact same ecological and physiological niche and therefore higher resource competition, rather than differing roles and lower competition if growing beneath a different species, such as manuka. The larger root systems for the seedling would help by increasing access soil resources which were already being utilized by the adult. Varied species composition allows each species to take advantage of different resources due to their different roles, and this type of energy partitioning is one reason high diversity ecosystems are sustained in nature (Dawkins and Krebs 1979, Wright 2002, Ives and Carpenter 2007, Hughes et al. 2008). It has been shown, however, that intraspecies competition is usually low when compared to interspecies competition (Stoll and Prati 2001), thus suggesting that escaping manuka is more important than moving away from other kanuka trees. This makes the benefit that ectomycorrhizal colonisation provides by improving root:stem ratio in the driest environments, beyond where manuka is a superior competitor, likely to be more valuable than growth gains for individuals near conspecifics. Also, late successional species are more susceptible to intraspecific completion issues than pioneer species, not considering the higher dispersal abilities of these species (Saha et al. 2014). So, competition between *K. ericoides* plants is most likely small, supporting by the fact that *K. ericoides* usually forms dense stands as an adult.

This experiment showed negative effects occurred from only using the soil taken from under an adult. Thus, a more likely cause of the reduced growth of the kanuka-soil is the presence of specific *K. ericoides* soil enemies or pathogens that were absent (or killed by the sterilization) in the other treatments. As is suggested by the Janzen-Connell model, host-specific pathogens and predators maintain spatial tree diversity by reducing seedling performance near conspecific adults (Clark and Clark 1984, Packer and Clay 2000, Casper and Castelli 2007, Petermann et al. 2008, McCarthy-Neumann and Kobe 2010). Also, the added stress of drought on plant physiology can increase the likelihood of disease infection due to reduced overall vigour of the plant (Desprez-Loustau et al. 2006). It is possible for plants to facilitate the growth of its own seedlings, as is the case for some southern beech species (McIntire and Fajardo 2011, Vogt et al. 2014), but when combined with ECM inoculation, this does not seem to be the case for kanuka. The reduced biomass of the seedlings from kanuka-soil may have also been driven by differences

in soil nutrient levels. There is a high heterogeneity in soil characteristics (Zhang et al. 2010, Croft et al. 2012), which may have led an unfortunate and accidental lack of soil resources due to site selection for the non-sterilized kanuka-soil group.

It must also be remembered this experiment tested AMF inoculation (control group) versus ECM and AMF inoculation (experimental group). Thus, the manuka-soil may have supplied a more suitable habitat for the AMF species (as discussed in Chapter 2), which could act as an amplifier to the ECM mutualism. So in areas of high soil moisture, the manuka edaphic features may have facilitated a stronger ECM effect, allowing *K. ericoides* to grow taller. Also, *K. ericoides* may have to grow taller more quickly to compete with manuka in wet habitat. This would lead to the allocation of resources to above ground biomass for seedlings grown in manuka-soil seen in the results. As manuka is considered as a prime competitor in wet surroundings (Stephens et al. 2005), utilizing the ECM mutualism benefits may allow *K. ericoides* to escape from competition of a very similar species.

4.5.3 ECM inoculation and water usage

ECM infection can contribute to delayed water stress (Pena et al. 2013). The water usage benefits of mycorrhizal presence have been found to be particularly pronounced under highly stressful conditions (Read and Boyd 1986). This was shown in this pot experiment by the ECM inoculated seedlings having significantly reduced daily water usage, especially under high water stress. As ECM associates with woody perennials, there is a wide range of habitats in which water may have seasonal availability. Summer drought and winter freezing are two common examples of when mediation of water stress may become essential to a plants survival (Lehto and Zwiazek 2011). ECM inoculation, while increasing the ability to access soil moisture, actively reduced the physical water usage by the plant, rejecting our supposition that access was the main pathway for mycorrhizal drought remediation. Ectomycorrhizal roots, as opposed to non-colonised roots, have been shown to have reduced hydraulic activity, which may conserve soil nutrient resources during prolonged periods of drought (Lehto and Zwiazek 2011). Reduction of water uptake reduces nutrient movement, meaning that under high water stress, ECM may facilitate an abatement of plant physiological activity to protect against increasing nutrient deficiencies which would result if normal metabolic activity was maintained during drought events (Lehto and Zwiazek 2011). Plants already reduce growth when a water shortage

occurs, and in this experiment, ECM seems to complement the ability of *K. ericoides* to endure and recover from drought conditions.

Considering current global climate change issues, the ability of ECM to function in drier ecosystems and mediate drought conditions, is an important mutualism to understand for the future of ecological functioning both worldwide and in New Zealand. As global temperatures rise and extreme drought events become more regular, the role of ectomycorrhizal mutualism can easily be seen becoming more crucial in maintaining ecosystems. By facilitating water access and usage, ECM mutualisms are diminishing the effect of climate changes and may allow currently dominant woody species to maintain their range or possibly expand into drier habitat. The larger effects of the mutualism seen at the lowest moisture levels suggest that this experiment did not measure the maximum possible benefit to be supply by ectomycorrhizal partnership. The wider application of drought mediation by mutualisms could help in range modelling and prediction under future climate calculations.

4.5.4 Conclusions

While the ECM mutualism did show positive effects on growth, overall this was limited possibly due to the timeframe of the experiment. The increase of root system size was dramatic and shows that ectomycorrhizal colonisation influences how plant resources are allocated at least during the establishment phase of *K. ericoides*. The reduction of water usage was impressive and shows the possible role of ectomycorrhizal inoculation in wider ecosystem functioning and environmental stress mediation. Overall, the effect of soil type on ECM colonisation affected above-ground biomass production. The manuka-soil caused taller plants to grow when compared to kanuka-soil. While ECM colonisation was variable in the biomass produced, *K. ericoides* received benefits (larger root systems, water stress mediation) that have been shown to increase levels of seedlings survival in their natural surroundings (Lehto and Zwiazek 2011).

Pot experiments by their nature are limited in their ability to reproduce nature. The assessment done in this thesis was incomplete. A more in-depth project would have studied the mutualism between *K. ericoides* and both arbuscular and ectomycorrhizae individually, allowing each interaction to quantified. This was not possible for this research due to time and financial constraints. The benefit of mycorrhizal mutualisms has been shown to be variable across many ecosystems and variables (Allison and Goldberg 2002, An et al. 2008, Cavender-Bares et al.

2009, Smith and Read 2010, Toth and Barta 2010, Macia-Vicente et al. 2012, Moeller et al. 2014), but the ubiquity of these relationships requires further examination to be understood and be properly incorporated into the general knowledge of biotic interaction.

Chapter 4 Final thoughts and future directions

Kanuka plays a fundamental role in New Zealand ecology as a pioneer and ecosystem facilitator. Mycorrhizal mutualists are ubiquitous biotic forces with far reaching ramifications for all levels of ecological interaction (Brundrett 1991, Dickie and Moyersoen 2008). This includes individual fitness, population development and maintenance, nutrient cycling, and ecosystem functioning (Read 1991, Hurst et al. 2002, Koide and Dickie 2002, Hoeksema and Kummel 2003, Read and Perez-Moreno 2003, Smith and Read 2010, Dickie et al. 2013). However, the variability and widespread nature of these relationships makes wide ecological understanding difficult. The mutualisms this tree species forms with both arbuscular and ectomycorrhizae helps it to adapt a wide range of abiotic factors and likely contributes to its ability to survive in difficult growing circumstances. The relationship it forms with ectomycorrhizal fungi makes it a unique woody species in New Zealand and increases its ecological influence in the successional sequence and ecosystem structure (Clements 1936). By evaluating the mutualisms this species forms across a hydrologic gradient, the tangible effects of these biotic interactions can be seen and possibly translated into ecosystem management decisions. Utilizing the benefits of mycorrhizal interactions can help to improve the quality and overall impact of environmental restoration by increasing the amount of initial success and expanding the ecosystems ability to cope with changing climatic conditions. This project has found that the dual-mycorrhizal nature of *Kunzea ericoides* makes it more successful across its realized range on the South Island of New Zealand.

This project focused on the seedling phase of *K. ericoides*. While this is an essential period of all plant life cycles, the adult mutualism relationship still needs exploration. The magnitude of mycorrhizal associations for mature plants still remains to be quantified and understood. Also, further study into the species of fungi will give a more specific view of where these mutualisms best function. This also applies to the newly described *Kunzea* species, which have not been evaluated for mycorrhizal association. The mechanism behind the dramatic decrease in water

usage needs to be further explored to understand which mechanisms are being employed by the mycorrhizae.

The study of mutualisms, not limited to mycorrhizal types, still has many factors to disentangle from the web of ecological interactions. Their role in ecosystem stability, diversity, maintenance, and functioning can still be further described (Bascompte 2009, Thébault and Fontaine 2010, White et al. 2012). Considering levels of climate change and the current extinction levels that the planet it facing, understanding how to promote these interactions may be essential in sustaining current and future ecosystem balance (Myers 2000, Butchart et al. 2010, Barnosky et al. 2011). Ultimately, understanding these ecological processes will provide scientists and land managers with the tools to best preserve and protect New Zealand's natural spaces for future generations.

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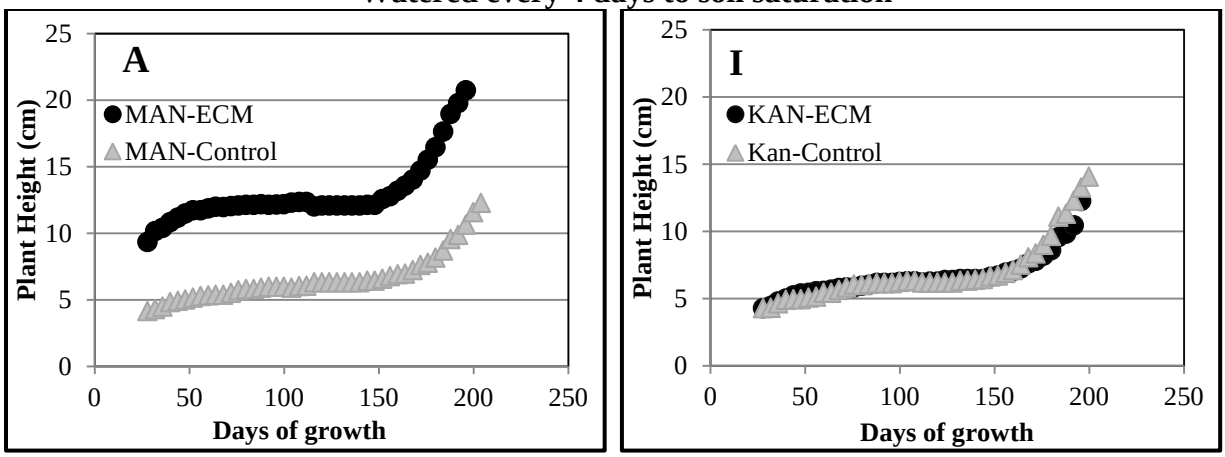
Appendix 1

This section contains the full graph accompaniment to the glasshouse experiment (see chapter 3 for full descriptions). These figures include seedling height over the growing period (Fig. 3.1).

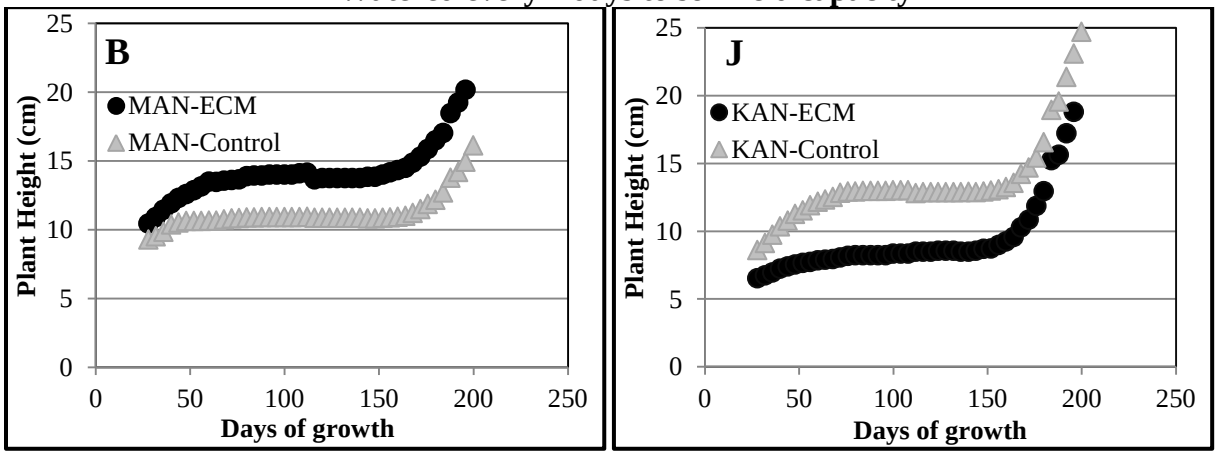
Figure 3.1A-P: Side-by-side comparison of height of *K. ericoides* seedlings height over the experimental period. The two groups were grown in soil taken from beneath manuka and soil taken from beneath kanuka. The experimental groups were inoculated with both ECM and AMF while the control group were only inoculated with AMF. Height measurements were taken at each scheduled watering date. Seedlings grown in manuka-soil on left (A-H), seedlings grown in kanuka-soil on right (I-P).

Watered every 4 days to soil saturation



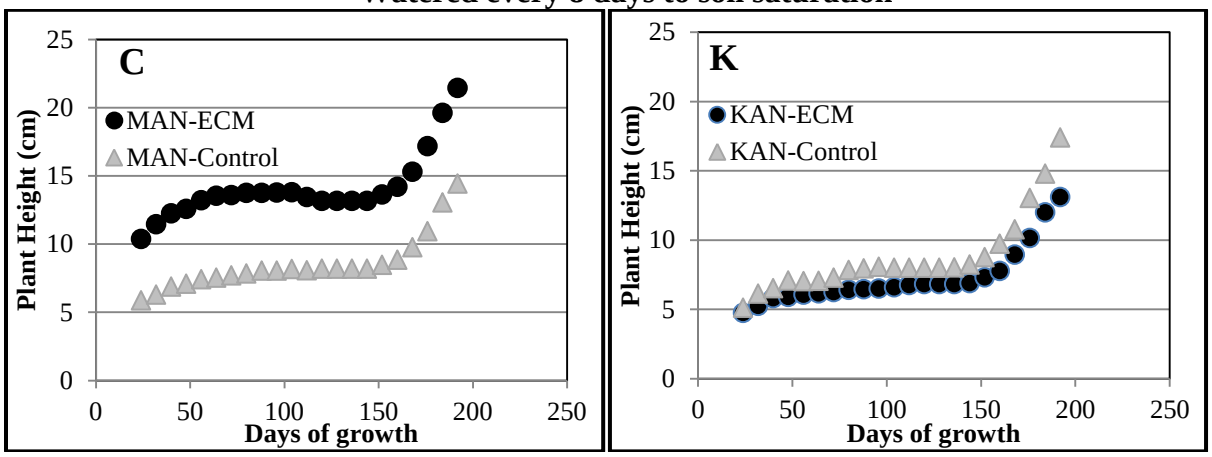
For manuka-soil seedlings (A), the ECM inoculated group grows significantly taller ($p<0.001$) than the control group.

Watered every 4 days to soil field capacity



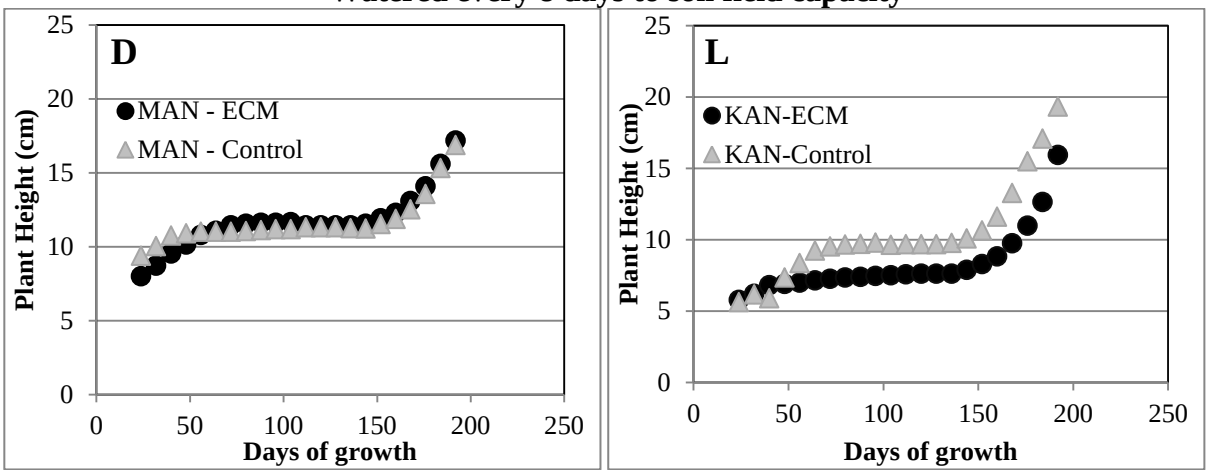
For manuka-soil seedlings (B), the ECM inoculated group grows significantly taller ($p<0.001$) than the control group. For kanuka-soil seedlings (J), the control group grows significantly larger ($p<0.001$) than the ECM inoculated group.

Watered every 8 days to soil saturation



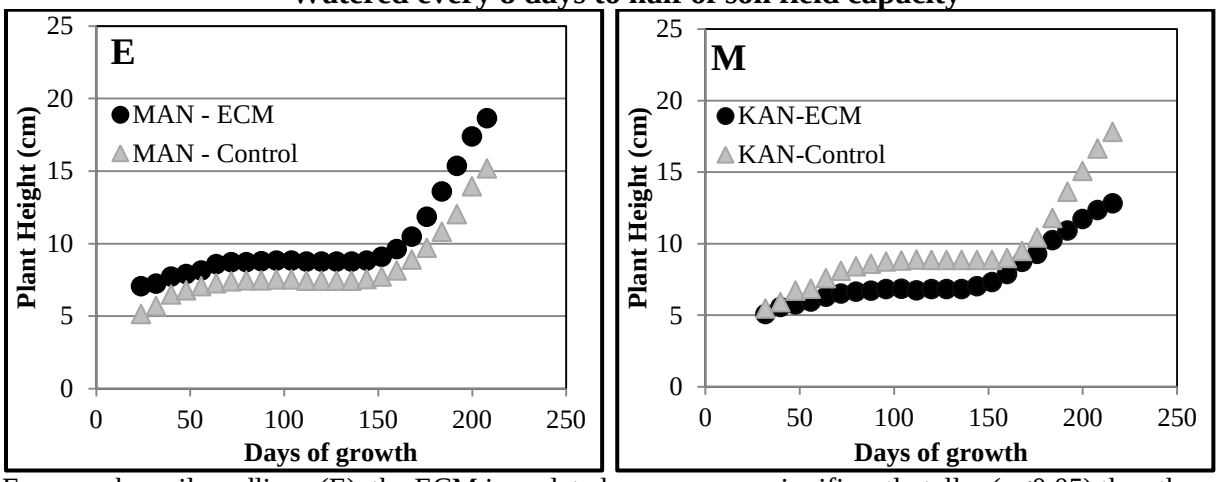
For manuka-soil seedlings (C), the ECM inoculated group grows significantly taller ($p<0.001$) than the control group.

Watered every 8 days to soil field capacity



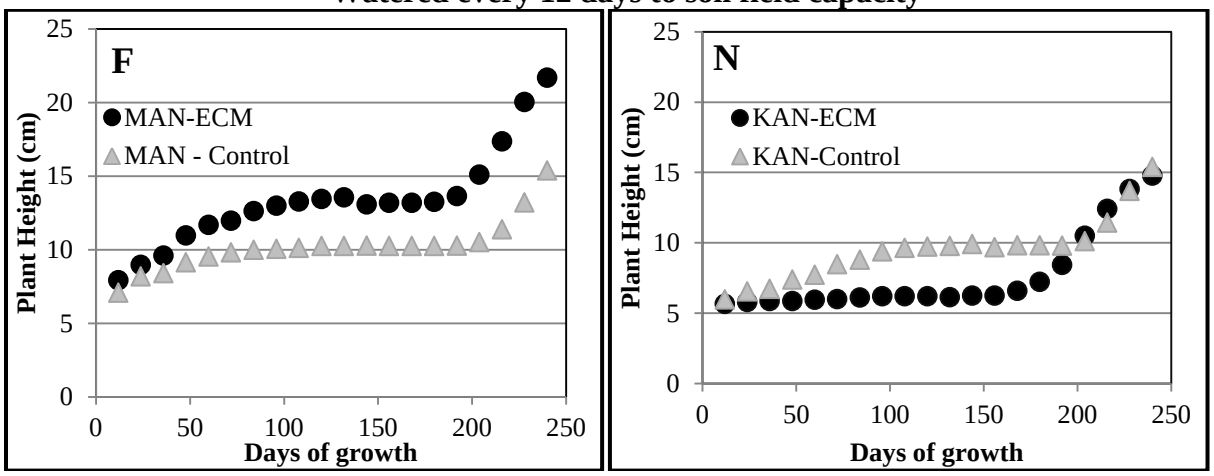
For kanuka-soil seedlings (L), the control group grows significantly taller ($p<0.05$) than the ECM inoculated group.

Watered every 8 days to half of soil field capacity



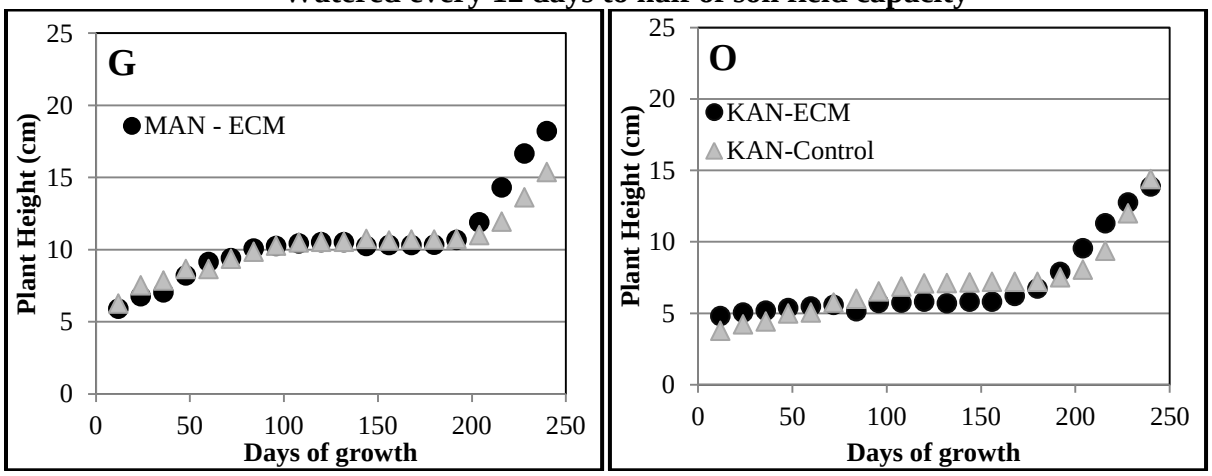
For manuka-soil seedlings (E), the ECM inoculated group grows significantly taller ($p<0.05$) than the control group. For kanuka-soil seedlings (L), the control group grows significantly taller ($p<0.05$) than the ECM inoculated group

Watered every 12 days to soil field capacity

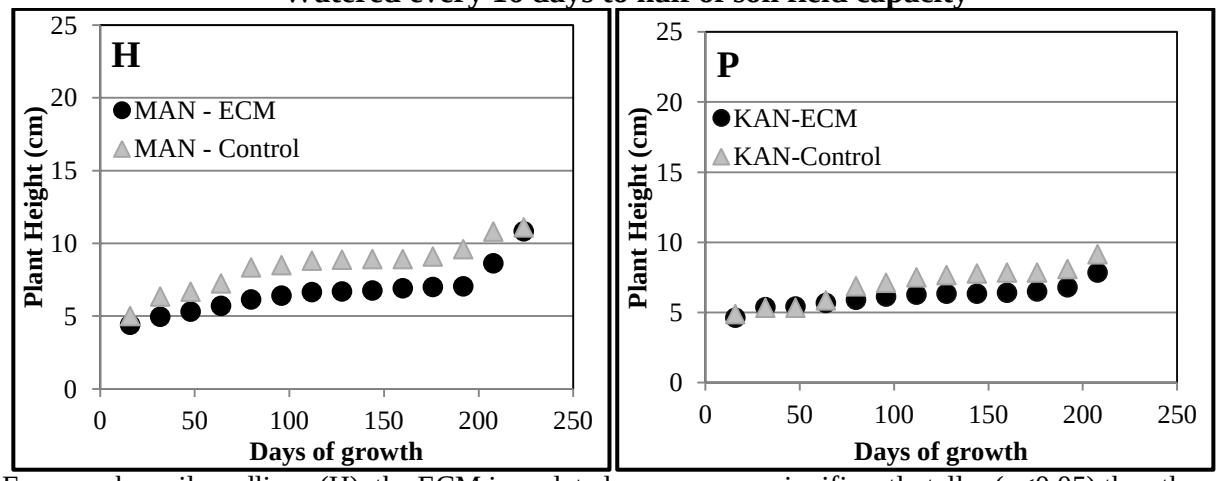


For manuka-soil seedlings (F), the ECM inoculated group grows significantly taller ($p<0.001$) than the control group. For kanuka-soil seedlings (N) the control group grows significantly taller ($p<0.05$) than the ECM inoculated group.

Watered every 12 days to half of soil field capacity



Watered every 16 days to half of soil field capacity

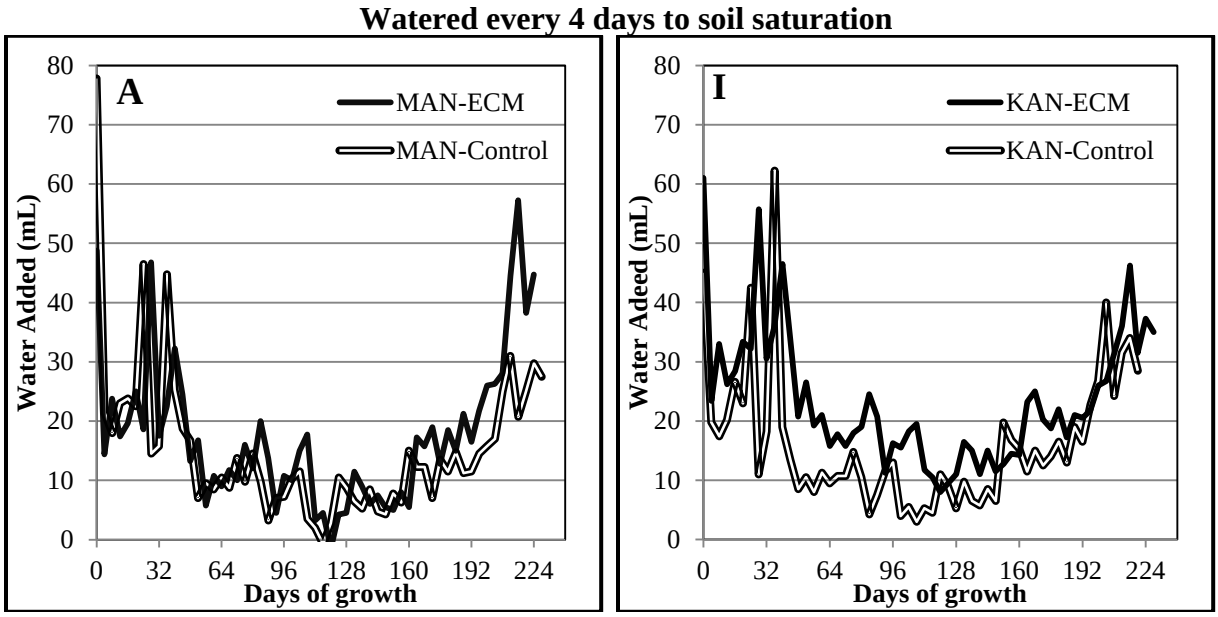


For manuka-soil seedlings (H), the ECM inoculated group grows significantly taller ($p<0.05$) than the control group.

Appendix 2

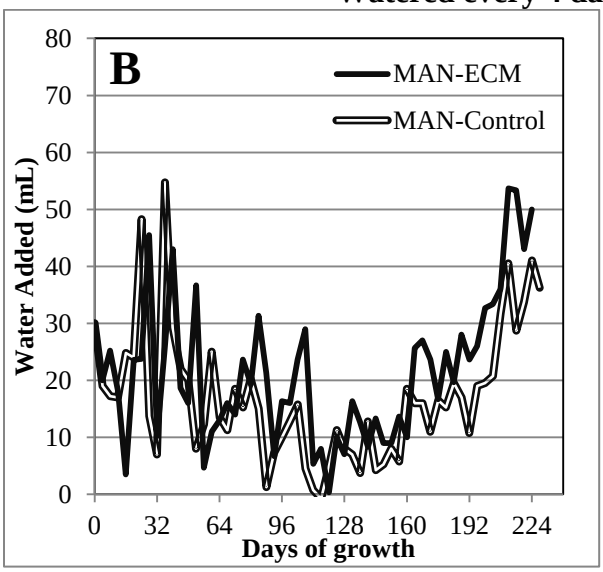
This section contains the full set of water usage graphs for each treatment group over the 9 month glasshouse experiment. ECM inoculation significantly reduced water usage for several treatment groups. This effect was especially pronounced when the seedlings were grown in drought conditions.

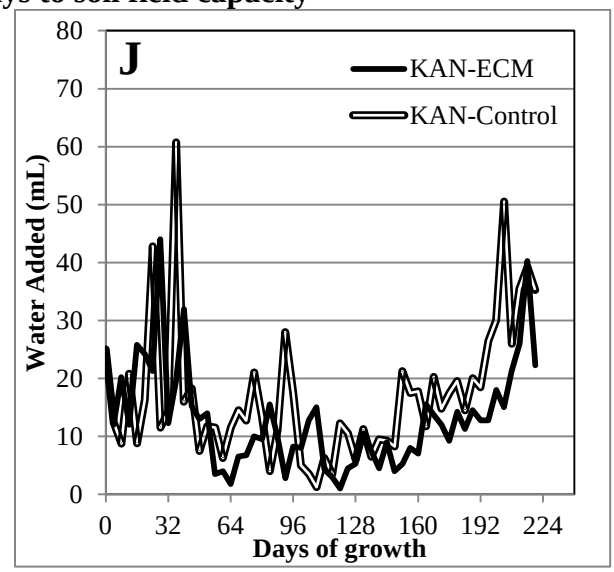
Figure 3.2A-P: Side-by-side comparison of water usage by *K. ericoides* seedlings grown in soil taken from beneath manuka and soil taken from beneath kanuka. The experimental groups were inoculated with both ECM and AMF while the control groups were inoculated with only AMF. Seedlings grown in manuka soil on the right (A-H), seedlings grown in kanuka soil on the left (I-P).



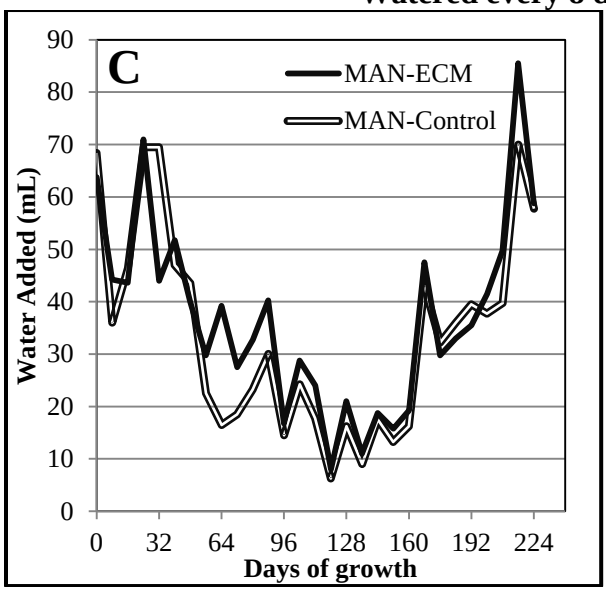
For kanuka-soil type (I) the control group had significantly lower ($p<0.05$) water usage than ECM inoculated seedlings over the growing period.

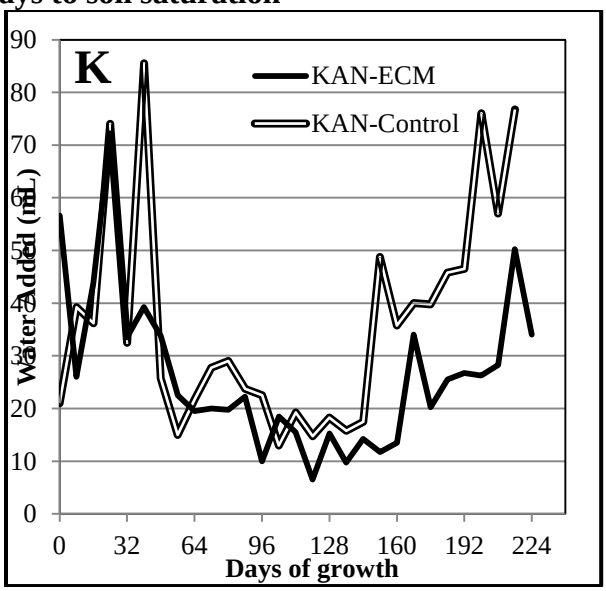
Watered every 4 days to soil field capacity





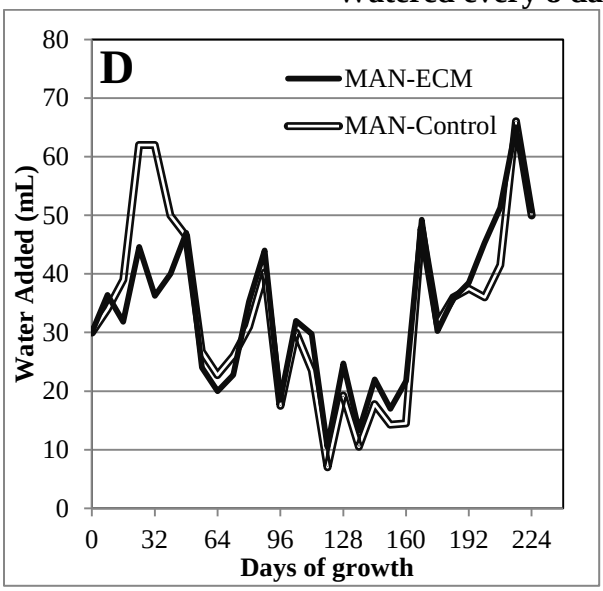
Watered every 8 days to soil saturation

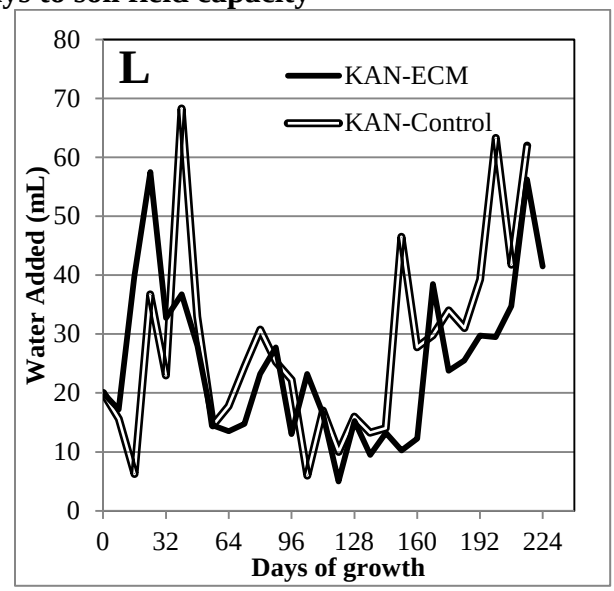




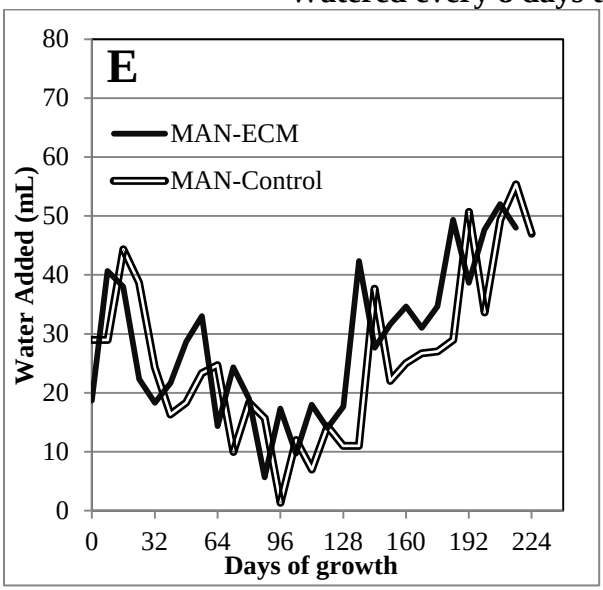
For kanuka-soil type (K), the ECM inoculated seedlings had significantly lower ($p<0.05$) water usage over the growing period.

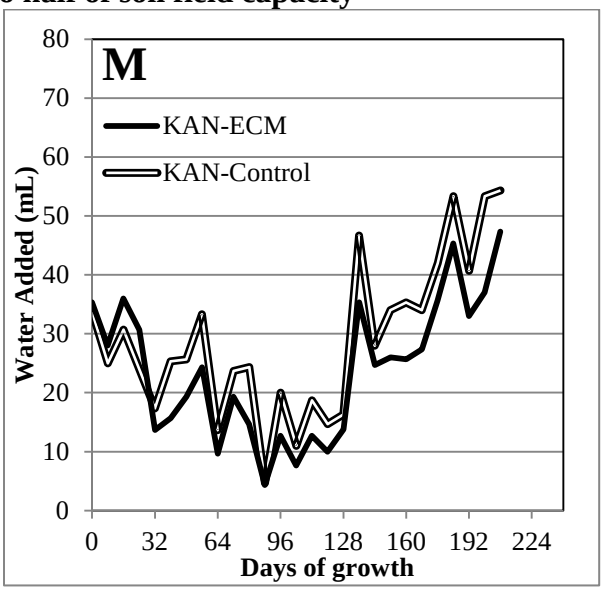
Watered every 8 days to soil field capacity



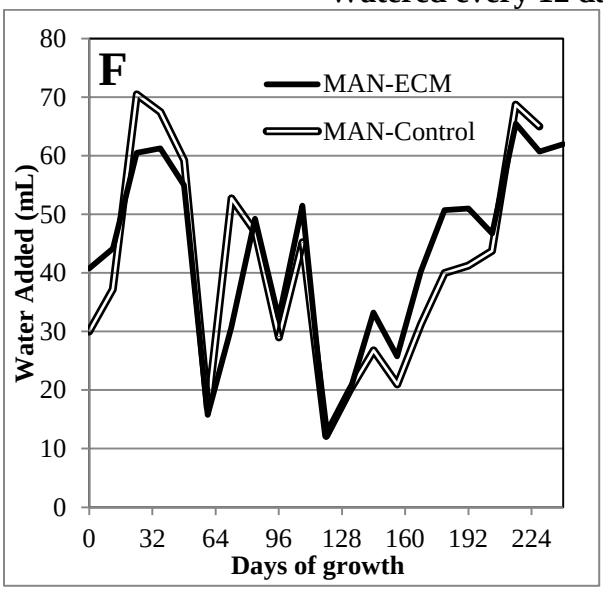


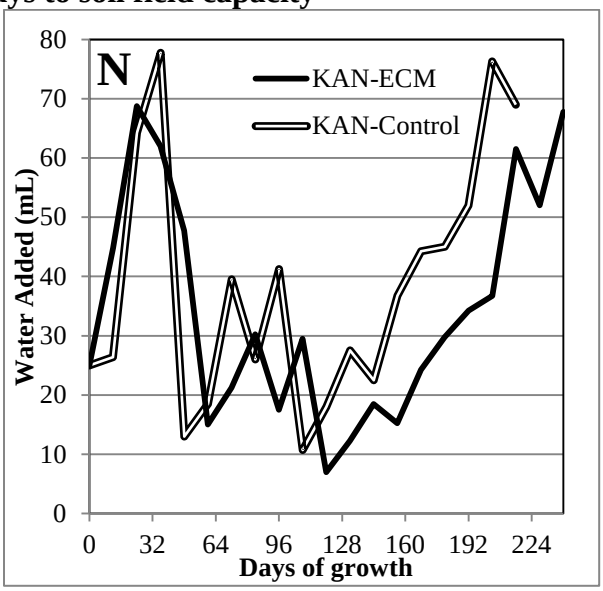
Watered every 8 days to half of soil field capacity



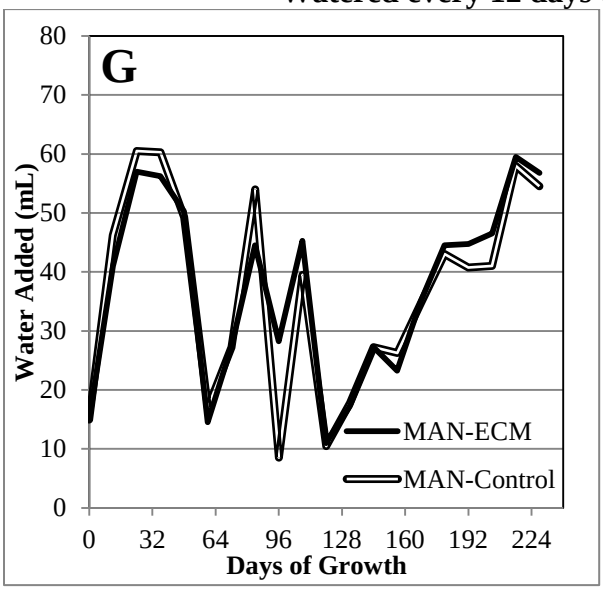


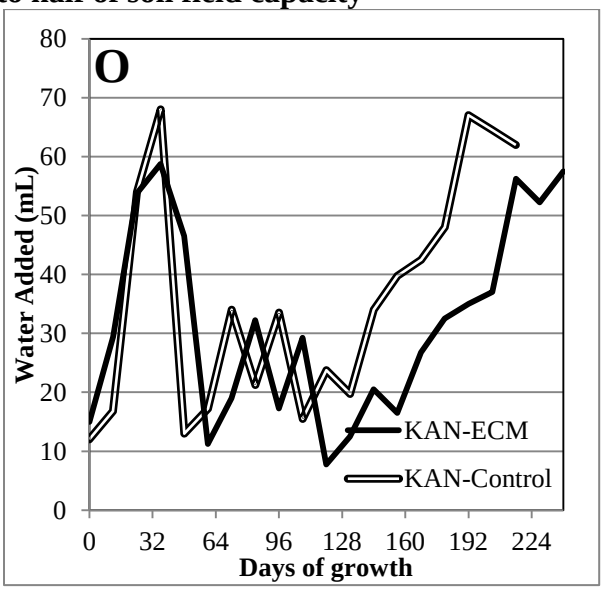
Watered every 12 days to soil field capacity



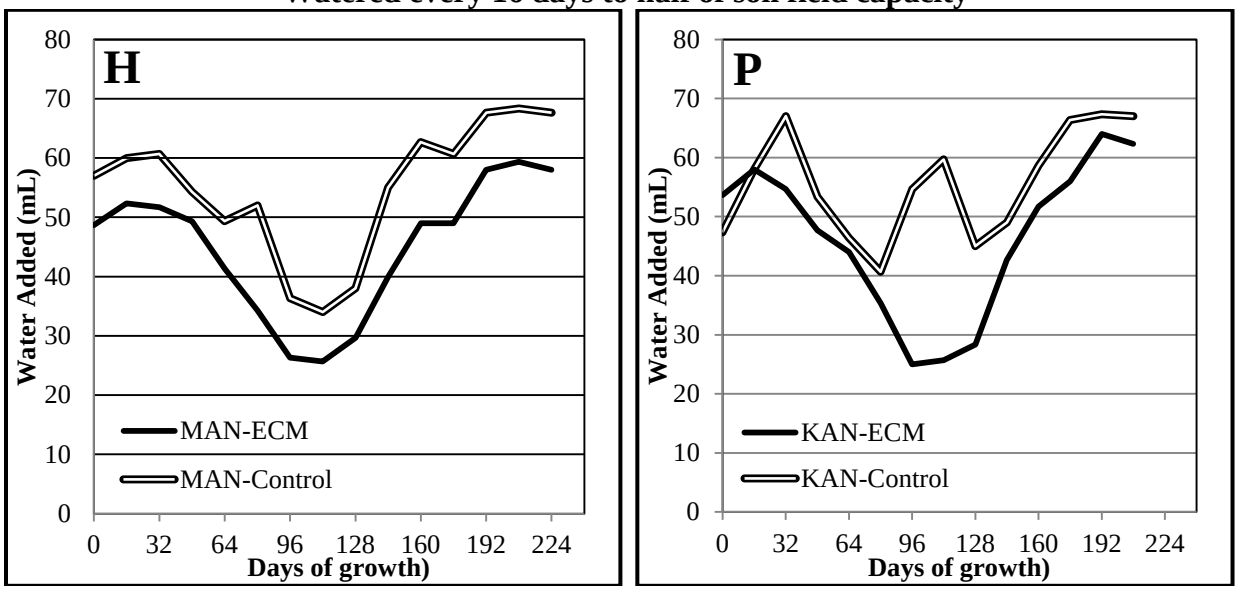


Watered every 12 days to half of soil field capacity





Watered every 16 days to half of soil field capacity



Both soils show significantly less ($p<0.05$) water usage over growth period for ECM inoculated seedlings.